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OTFRID FOERSTER 1873-1941

AN APPRECIATION

On August 11th, 1941, word reached the United States via Switzerland that Professor Otfrid Foerster had died in Breslau, Germany, on June 15th. Dr. Foerster had written to us during the preceding winter from a sanatorium in Switzerland, where he and his wife were both ill with tuberculosis. Frau Foerster survived her husband for only one day. A letter from his sister says: "So, they have both been buried together. All Breslau took part in the burial. There was a speech of the Protestant clergyman first, then: the Rector of the University, a representative of the town council, his successor Professor von Weizsäcker, who had come from Heidelberg at Easter (Internist); then two doctors from Hamburg and Frankfurt, finally the Oberarzt Dr. Stender. They all praised Otfrid's value to the utmost and the appreciation of him was really marvelous. At the last came the Roman Catholic priest for Marthe."

To the many neurologists, neurosurgeons, physiologists and anatomists of the English-speaking countries, who have been to Breslau in the past twenty years, this description will recall much of the city, the hospital and the associates which form the background of a man who had become the foremost teacher and most learned authority in the field of the neurological sciences and one of the strongest personalities of his time in medicine.

Otfrid Foerster* was born at Breslau, November 9th, 1873. His early years were spent at Kiel where his father held the chair of Philology, but he later had schooling at the gymnasium at Rostok and at the Maria Magdalene Gymnasium in Breslau receiving his diploma in 1892. Between 1892 and 1896 he studied medicine at the Universities of Freiburg, Kiel and Breslau, taking his state medical qualifications at Breslau during the winter semester of 1896–97. His inaugural dissertation entitled *Quantitative Untersuchungen ueber die agglutinirende und baktericide Wirkung des Blutserums von Typhus-kranken und-Reconvalescenten* written at Breslau, was defended on May 20th, 1897.

This dissertation is the only paper published by Foerster† which was not directly connected with matters of the nervous system. During his student years he had spent some months in the Psychiatric Hospital at Lüben where Kraepelin had first worked and he had come under the influence of Wernicke in the Medical School at Breslau. At the suggestion of the latter, he went in 1897 to Paris and remained there for two years as a pupil of Déjérine, attending at the same time the clinics of Pierre Marie, and of Babinski. The summers of these years were spent at the clinic of Professor Frenkel in Switzerland, and in 1899 he was sent to the United States to demonstrate

* In his early life Dr. Foerster spelt his given name "OTFRIED." In 1903 he dropped the 'e' and adopted the spelling of OTFRID.

† A complete list of Dr. Foerster's writings follows this appreciation.

Frenkel's new "Uebungstherapie" for tabes. His memories of this early trip were always vivid; he stayed in Tarrytown with an American gentleman who was representative for a firm buying French and German wines, and it was here, he recalled, rather than in Germany that he learned about the Rhenish wines; here also he met John D. Rockefeller, senior, who, Foerster related, spoke excellent German. Just before his return to Paris he visited Washington and, because funds by then were low "and because I liked them," he lived for some days on bananas! In 1899 he returned to Breslau and entered the Clinic of Wernicke where he collaborated with him in his published *Atlas* of brain sections issued in 1903.

His extraordinary memory and ability to apply and order his knowledge were early recognized; it was said that as a boy and young man his knowledge of botany, geology and other natural sciences was remarkable. An accomplished linguist, schooled by his father in the use of words, he had learned to read, speak and write faultless English as well as French and Italian and he had a fair grasp of Polish, Russian and the Scandinavian languages.

About 1905 he began to study the English neurophysiological literature, beginning with David Ferrier and following through Beevor, Horsley, Schäfer and, finally, Sherrington who, as he often said, influenced his thinking more than any other writer. Students who went to Breslau often saw a well-worn copy of *The integrative action of the nervous system*, and he had read and closely analyzed all of Sherrington's later papers. When Prof. E. G. T. Liddell, who with Sherrington had first described stretch reflexes, met Foerster in 1930, Foerster remarked that their papers on the myotatic reflex had clarified for him one of the principal problems of neurology.

His study at home where all his writing was done (before 11 A.M., when he went to the hospital, or late at night), contained the books for which he cared most. They were notably few: the complete works of Helmholtz, Goethe and Schiller, the collected works of Hughlings Jackson, Sherrington, and Harvey Cushing. But his reprints were beyond counting. They lay in large and apparently chaotic piles on tables, shelves and on the floor. But it only took him an instant to find among them the particular reference which he might need to illustrate a point.

The chronological sequence of his three hundred published works, given in the bibliographical list which follows, will indicate the progression and order of his work, the breadth of his knowledge and the tremendous industry which enabled him, an active clinician and a surgeon with a private practice, to pursue investigations and to write (always in his own hand), such compendia as are represented by his articles in the *Handbuch der Neurologie*.

Throughout the forty years of his active professional life one perceives two dominating characteristics: his power of observation and analysis, and his capacity to apply fundamental knowledge toward the relief of symptoms —this at a time when clinical neurology was still under the influence of the purely diagnostic schools of Paris and of Queen Square, and before the era

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of neurosurgery. In this field of neurology where therapy had been often unconsidered, Foerster's physiological insight led directly to the use of surgery, in the relief of intractable pain through chordotomy, and of spasticity through dorsal root section. His analysis of the distribution of the sensory dermatomes arose from his use of the latter procedure. Diagnosis of cortical tumors and of focal scars in epileptics by stimulation and subsequent ablation, under local anesthesia, led to his equally fastidious analysis of functional localization in the cerebral cortex. These and his clinical studies on basal gangliar disease all bear the mark of an originality and courage almost without parallel in clinical medicine.

Before the onset of the first world war he had as neurologist directed the surgeons, Tietze and Küttner, in the execution of surgical procedures. In 1914 when general surgeons had become scarce, he began, although more than forty years of age, to do his own neurosurgery. Self-made as a surgeon he always lacked refinement of technique, but his results were surprisingly good because of his remarkable speed and manual dexterity. Although hemostasis and even asepsis were often crude, the operations for nerve sutures and cord sections were carried out as perfect anatomical dissections.

After the last war Foerster published many papers on war injuries, and then by logical progression, and through fortunate association with Gagel as neuropathologist and Altenburger as neurophysiologist, the bulk of his work was diverted into diagnosis of cerebral tumors and to electrophysiological analysis. The neurosurgical operations in his hospital were done by Dr. Stender during the last few years, although Professor Foerster remained Director of the *Neurologisches Forschungsinstitut* until 1941.

He was as remarkable a teacher and leader as he was physician. For many years as President of the *Gesellschaft deutscher Nervenärzte*, his opening addresses were masterpieces of descriptive history of the development of German medicine. His lectures, into which he put much time and great effort, were models of clarity and precision in whatever language he happened to be speaking. His presentations were always highly illustrated as were his published works. We have known him to show as many as 160 lantern slides during a single lecture, their sequence being unfailingly memorized and the order of his thought undisturbed by their presentation.

His contacts with other countries were extensive. He visited the United States four times: first in 1899, as above mentioned, and then in 1912 when he addressed the American Association of Physicians and Surgeons at the instigation of Franklin Martin. In 1914 he came again and spoke before the American Medical Association at Atlantic City. His fourth visit was in 1930. From a distance he had long admired the work of Harvey Cushing, and when asked by Dr. Cushing to act as Surgeon-in-Chief *pro tem* at the Peter Bent Brigham Hospital in Boston, he immediately accepted. The result of this exchange was far-reaching and both Cushing and Foerster referred to it in later years with particular satisfaction. Foerster went frequently to England, and in 1937 the Society of British Neurological Sur-

geons, which includes in its membership many neurosurgeons of continental Europe, held its annual meeting in his Neurological Institute (now renamed 'Otfrid Foerster Institut') at the Wenzel Hanke Krankenhaus in Breslau.

During Professor Foerster's last years his work was restricted to some degree by increasing tension within his country. He was under surveillance both because he had for a time been physician to Lenin in Moscow, and because his wife was partly Jewish. By 1936 he, with many others, ceased subscribing to all journals outside Germany, and in 1938, when he was asked to represent German clinical neurology and neurophysiology on the Advisory Board of this *Journal*, he felt unable to accept the appointment.

Yet for the fifteen years preceding 1939 students from the United States, Great Britain, Canada, Australia, South America, China and Japan; from Scandinavia, Rumania, Holland, France and Italy had found in remote Breslau a man already cognizant of all that was significant in neurology in each of their countries, and one who was usually personally acquainted with their leaders. From him they gained much factual knowledge and much of the history and development of their science. They saw a neurosurgeon self-trained in surgery; a neurologist with few equals in his knowledge of anatomy and physiology; a gifted teacher,—master of many languages,—and artist in the use of his own, and a physician who inspired a deep sense of personal devotion in his patients and students. They returned to their homes, deeply influenced by contact with this man whose life had been inflexibly shaped and disciplined to follow one ideal and who, through incessant effort and through rigid economy of time, and even of friends, has left an indelible impression on the clinical neurology of this generation.

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* Copies of this can be found in the libraries of Dr. John F. Fulton, Yale University School of Medicine, New Haven, Connecticut, Dr. Paul C. Bucy, Chicago, Illinois, and Dr. C. G. de Gutiérrez-Mahoney, Vanderbilt University School of Medicine, Nashville, Tenn.

† This Oration is transcribed in full in: FULTON, J. F. Arnold Klebs and Harvey Cushing at the 1st International Neurological Congress at Berne in 1931. *Bull. Hist. Med.*, 1940, *8:* 332-354.

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protrude into the paraffin while still remaining surrounded by a thin film of saline, i.e. without breaking at any point the paraffin surface. In Fig. 2. a fine film of saline should be shown between the fibre M and the paraffin P. With successive movements of the electrode the interface position with regard to the muscle fibre is unaltered. One leading electrode thus made contact with the fibre, while the other lead was kept in the saline, forming a large indifferent lead. Small movements (50–100μ) of the leading electrode along the muscle fibre were made by means of a micrometer adjustment under direct microscopic observation. Accurate electrode shifts were only possible when the muscle fibre had been freed from loose connective tissue.

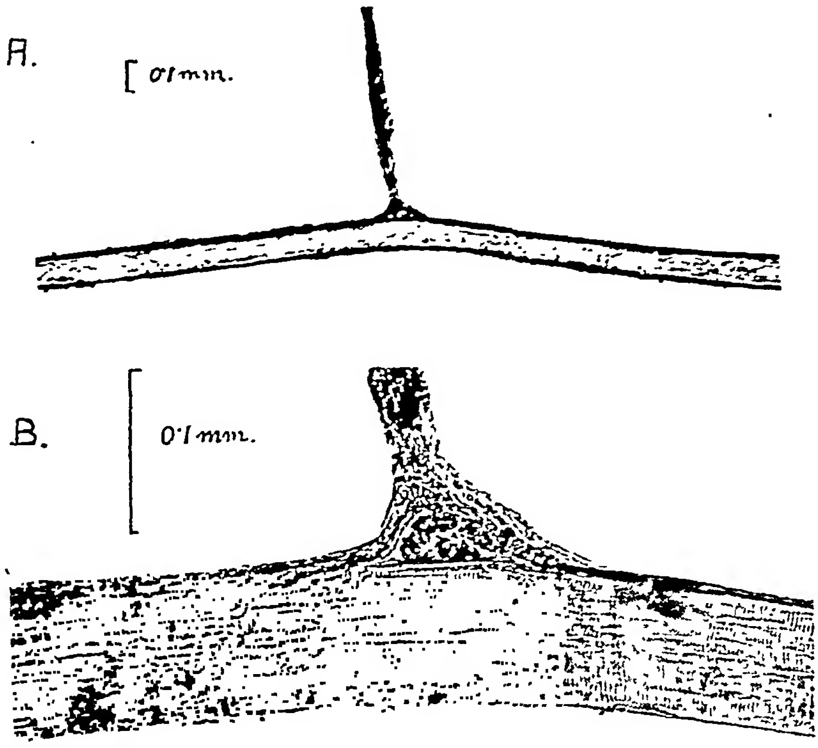

FIG. 1. Microphotograph of a living single muscle fibre with its nerve supply. A, lower. B, higher magnification. Dark area to the right of the nerve-entry is a small air-bubble.

In frog's muscle the end-plate extends over about 100μ fibre length, hence in order to record selectively from such a region, the recording electrode must make a very good and accurate contact and it must be insulated elsewhere. This was done most effectively by embedding a platinum wire of 50μ diameter in a fine glass hook (about 300μ thick), so that only a small area of the platinum was uncovered on the inner side of the angle of the hook. When the hook pulls the muscle up at the paraffin-saline interface the platinum electrode will thus make a good contact with a small area of the muscle while the surrounding glass will largely prevent it from leading from adjacent regions of the muscle (Fig. 2).

A single nerve-muscle fibre preparation survived under experimental conditions in the interface for 6–8 hours and sometimes was kept as long as 24 hours without appreciable deterioration. Isolated single muscle fibres, without their innervation, have been kept in Ringer by Ramsay and Street (1941) up to ten days and gave normal propagated twitches if stimulated directly. In paraffin the muscle fibre frequently ceased to conduct after 1–2 hours. So far 25 successfully dissected preparations have been experimented on.

ELECTRIC POTENTIAL CHANGES AT AN ISOLATED NERVE-MUSCLE JUNCTION

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IN RECENT studies of neuromuscular transmission one of the main difficulties was the scatter of the nerve-muscle junctions. This was partly overcome by using a strip preparation of the cat's soleus (cf. Eccles and O'Connor, 1939) which contains nerve-muscle junctions restricted to 1–2 mm. of muscle length. Further investigations (Eccles, Katz and Kuffler 1941a, b; Eccles and Kuffler 1941a, b) were done on this strip-preparation and on frog sartorius which contains discrete foci of nerve endings (Katz and Kuffler, 1941).

Although a sharp focus of nerve-muscle junctions would give an electric response similar to that of a single element, there are obvious advantages in recording from an isolated single neuro-muscular junction. The main objects of this investigation on the isolated nerve-muscle fibre preparation (cf. Kuffler, 1941) are (i) to record the potential change at the small area of the junction itself, as distinct from the rest of the fibre; (ii) to find the relationship of this potential to the origin of the muscle spike potential.

METHODS

Different muscles in frog (*Hyla aurea*) were tried, such as M. glosshyoideus, the leg-skin muscle, M. semitendinosus and other leg-muscles. Finally the M. adductor longus was found the most suitable. This muscle is 2–3 cm. long, has parallel fibres about 80μ in diameter and comparatively little connective tissue.

The muscle is removed with 2–3 cm. nerve attached and put into a glass-chamber filled with saline. This chamber is placed on a microscope stand with both direct and transmitted illumination. The dissection is done under a binocular microscope and a magnification of 10–30 is used. During the whole dissection the muscle is kept in saline while the tendinous ends are gripped by screw-adjustable forceps which hold the preparation taut. With M. adductor longus the proximal end is left attached to the insertion at the symphysis. After removing the connective tissue sheath with fine scissors the muscle is dissected with thin needles and silver-steel knives with fine edges and sharp points. The course of the nerve down to its finer branches can be followed up easily and all muscle fibres are removed except those innervated by one of these fine branches. When about 10 fibres are left in a muscle-bundle, fibre after fibre is dissected away, care being taken not to injure the nerve, until eventually a single muscle fibre is left with its innervation intact. (Microphotograph Fig. 1.)

As a rule the fibre need only be dissected clean over a few millimeters each side of the nerve entry. Slight injury of the muscle fibre such as touching with the needle, excessive pulling when freeing it from adjacent muscle fibres and connective tissue will damage the fibre easily and render it inexcitable in a few minutes. Such an injury can be seen under the microscope and at these places the protoplasm retracts from the sarcoplasma, becomes opaque and seems to break up. The injury spreads very quickly along the muscle fibre.

Two methods of recording were used. (i) The fibre was either lifted up into paraffin floating on the saline (Hodgkin, 1938) or (ii) was brought up to the saline-paraffin interface where the recording was done while the nerve was stimulated in the overlying paraffin. This latter method had the advantage that drugs such as curarine could easily be applied to the preparation through the saline and there was better contact between recording electrode and fibre, because this was pressed against the electrode by the interfacial tension of the saline-paraffin junction. As shown in Fig. 2 the muscle fibre was lifted up so as to

quick positive wave. Similar findings were obtained in the cat (Eccles and O'Connor, 1939; Eccles *et al.*, 1941b) and are due to the leading conditions (see above). The initial positive deflexion makes it difficult to interpret accurately potentials recorded some distance from the n.m.j.

Thus it is evident from Fig. 3a, b, c, d, that a nerve impulse sets up a potential at the n.m.j., the "end-plate potential" (henceforth e.p.p.), which reaches its peak in about 0.5 msec. and decrements very rapidly along the muscle fibre. No accurate data about this electrotonic decrement can be given owing to distortions due to the leading conditions (but see Fig. 3). The rapid spatial decrement would be expected for the electrotonic spread of such a brief potential change (cf. Bogue and Rosenberg, 1934).

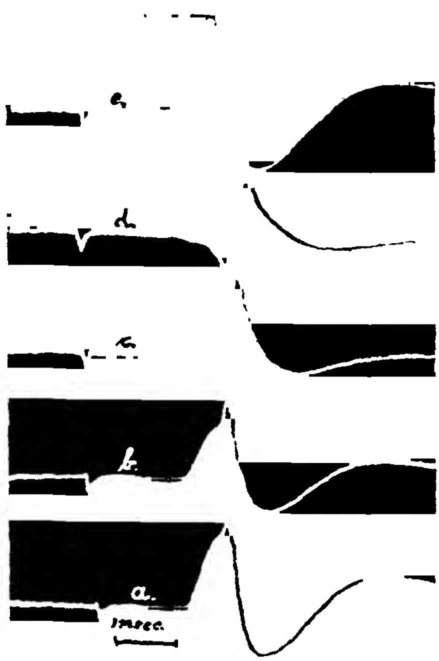

FIG. 3. Recording at different positions on the muscle fibre. a, at the nerve muscle junction; b, c, and d, at 80, 230 and 500μ distance from the junction. e, after careful placement of the electrode on the end-plate, no spike action potential is observed (see text).

In the earlier experiments potentials similar to Fig. 3c were usually recorded at the n.m.j. However, with improvement of the dissection technic the muscle fibre was freed from nearly all surrounding tissue and it was possible to obtain a much better contact of the recording electrode. Under such conditions and with careful placing of the electrode right on the motor endplate it was often found that the e.p.p. was so large that no spike could be detected rising above it (Fig. 3e).

The potential changes beyond the spike summit (cf. Fig. 3 and later figures) cannot be interpreted until the interaction with antidromic muscle impulses has been investigated.

The simplest way of recording from a single muscle fibre would be in paraffin oil, as was done by Hodkgin (1938) for single nerve fibres. However two main difficulties have not yet been overcome. (i) In the absence of interfacial tension, the fibre is not pressed against the electrode and, therefore, the contact is not well defined. Moreover, when the preparation is lifted into paraffin, some saline still adheres to it especially round the nerve entry, forming a drop of varying size. (ii) Little droplets along the fibre probably account for "false-leads" which complicate the records by introducing small diphasic waves similar to those described by Bishop, Erlanger and Gasser (1926) and Bishop and Gilson (1929). This latter difficulty could frequently be overcome by keeping the recording leads close together.

The stimulating and recording apparatus was the same as described by Eccles, *et al.* (1941b). For recordings in paraffin a preamplifier of low input capacity was added, so as to minimize distortions arising on account of the high input resistance. A rectangular input through a 2 MΩ input resistance then reached 90 per cent deflexion in 0.15 msec.

Results

A. The action potential at the nerve-muscle junction and some distance away from it

For recording from the single fibre in the interface similar conditions obtain as with the 'strip'-preparation of the cat (cf. Eccles and O'Connor, 1939, p. 49), or with an active nerve fibre in a large volume of a conductive medium (Bishop, 1937).

Figure 3a shows an action potential set up by a single nerve impulse when, using microscopic observation, the recording electrode is placed on

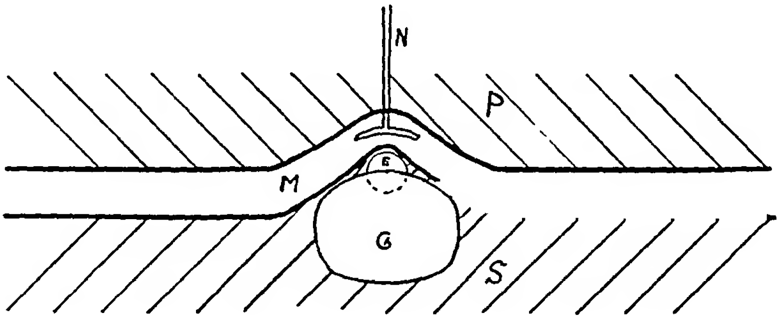

FIG. 2. The recording system at the paraffin-saline interface. P, paraffin oil; S, saline; G, glass rod (shown in transverse section) in which the platinum wire electrode E, is embedded; M, muscle fibre; N, nerve.

the neuro-muscular junction (n.m.j.). The potential rise is composed of two phases. The first component rises in about 0.5 msec. to 90 per cent total potential height, and the second smaller part follows after a slight delay. When the recording electrode is moved 80μ away (Fig. 3b), the latent periods of the two components are not appreciably altered, but the first is smaller and the second shows a larger rising phase. This differential effect on the size of the two components is much increased by a further movement to 230μ from the n.m.j. (Fig. 3c), and in addition the peak of the second component is delayed by 0.3 msec. Recording at progressively larger distances from the n.m.j. shows a corresponding increase in this delay. The spike-like second component is thus shown to be due to a propagating muscle impulse, the speed of propagation being about 2 m. per sec. On the other hand the latent period of the initial potential is not altered when recorded 80 or 230μ away from the junction, but its rate of rise is diminished. This indicates that it is due to a local potential generated at the n.m.j. and spreading from there electrotonically along the muscle fibre.

At 0.5 mm. distance (Fig. 3d) from the n.m.j. the initial potential is inversely recorded, while the negative phase of the spike is preceded by a

quick positive wave. Similar findings were obtained in the cat (Eccles and O'Connor, 1939; Eccles *et al.*, 1941b) and are due to the leading conditions (see above). The initial positive deflexion makes it difficult to interpret accurately potentials recorded some distance from the n.m.j.

Thus it is evident from Fig. 3a, b, c, d, that a nerve impulse sets up a potential at the n.m.j., the "end-plate potential" (henceforth e.p.p.), which reaches its peak in about 0.5 msec. and decrements very rapidly along the muscle fibre. No accurate data about this electrotonic decrement can be given owing to distortions due to the leading conditions (but see Fig. 3). The rapid spatial decrement would be expected for the electrotonic spread of such a brief potential change (cf. Bogue and Rosenberg, 1934).

In the earlier experiments potentials similar to Fig. 3c were usually recorded at the n.m.j. However, with improvement of the dissection technic the muscle fibre was freed from nearly all surrounding tissue and it was possible to obtain a much better contact of the recording electrode. Under such conditions and with careful placing of the electrode right on the motor endplate it was often found that the e.p.p. was so large that no spike could be detected rising above it (Fig. 3e).

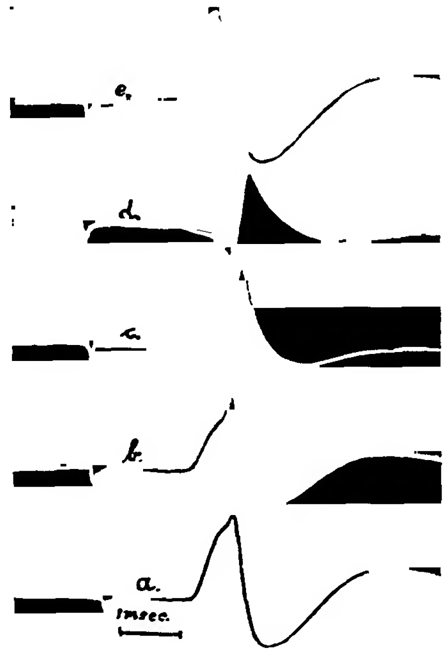

FIG. 3. Recording at different positions on the muscle fibre. a, at the nerve muscle junction; b, c, and d, at 80, 230 and 500μ distance from the junction. e, after careful placement of the electrode on the end-plate, no spike action potential is observed (see text).

The potential changes beyond the spike summit (cf. Fig. 3 and later figures) cannot be interpreted until the interaction with antidromic muscle impulses has been investigated.

The simplest way of recording from a single muscle fibre would be in paraffin oil, as was done by Hodkgin (1938) for single nerve fibres. However two main difficulties have not yet been overcome. (i) In the absence of interfacial tension, the fibre is not pressed against the electrode and, therefore, the contact is not well defined. Moreover, when the preparation is lifted into paraffin, some saline still adheres to it especially round the nerve entry, forming a drop of varying size. (ii) Little droplets along the fibre probably account for "false-leads" which complicate the records by introducing small diphasic waves similar to those described by Bishop, Erlanger and Gasser (1926) and Bishop and Gilson (1929). This latter difficulty could frequently be overcome by keeping the recording leads close together.

Figure 4a shows an observation at the n.m.j. in which the initial e.p.p. attains only about 40 per cent of the total action potential height. Figure 4b is a record taken in the interface at a comparable position. The relative sizes of the e.p.p. and spike and the periods of rise are not appreciably different from Fig. 4a, though as a rule the time course was a little slower in paraffin. Thus it appears from the recordings in paraffin, that the rising phase at the n.m.j. is not appreciably distorted when leading at the paraffin-saline interface. Action potentials up to 100 mV have been obtained in paraffin.

B. THE ACTION OF CURARINE

Curarine was added to the saline-bath so that it reaches the preparation after gradual diffusion. In this way it usually took 20–30 min. to exert its full effect on the junction. During this time action potentials could be recorded at various stages of curarization as shown in Fig. 5 and 6. A final concentration of 1–2μ mol per l. of curarine chloride is generally sufficient for a complete block of neuromuscular transmission.

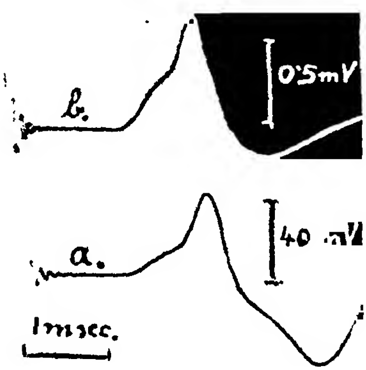

FIG. 4. a, diphasic action potential at the end-plate region, recorded in paraffin oil; b, action potential recorded in the interface with corresponding position of the recording electrode. Note the difference of potential scale: 40 and 0.5 mV. respectively.

Figure 5 shows a series of observations during curarization where the recording electrode is placed as near as possible to the n.m.j., the e.p.p. reaching a little over 70 per cent total potential height (Fig. 5a). The main actions of curarine are seen to be (i) progressive diminution of rate of rise and total height of the e.p.p., but no appreciable effect on its latent period; (ii) a progressive delay in the latent period of the spike, but no significant change in its height; (iii) finally as shown in Fig. 5e disappearance of the spike, leaving behind a pure e.p.p.

Thus the initial e.p.p. can be diminished from over 70 per cent total potential height in Fig. 5a to about 40 per cent in Fig. 5d and still a spike arises. With a slight further diminution (compare Fig. 5d and e), the absence of a spike shows that no propagated muscle impulse is initiated.

In all experiments where an e.p.p. of over 90 per cent total potential height was obtained, the e.p.p. diminished to 40–50 per cent during subparalytic curarization. Together with the reduction of the initial e.p.p. the residual end-plate negativity after the spike is greatly diminished, suggesting that in the non-curarized muscle, part of the e.p.p. outlasts the spike (cf. Eccles and Kuffler, 1941b). The time course of this surviving e.p.p. cannot be established accurately from the recordings in the interface.

As would be expected, once the spike is set up, it reaches an absolute height similar to the pre-curarine state, but variations within 15 per cent have been observed. In some experiments the latent period of the e.p.p. also varied, while no temperature change occurred. This is possibly due to

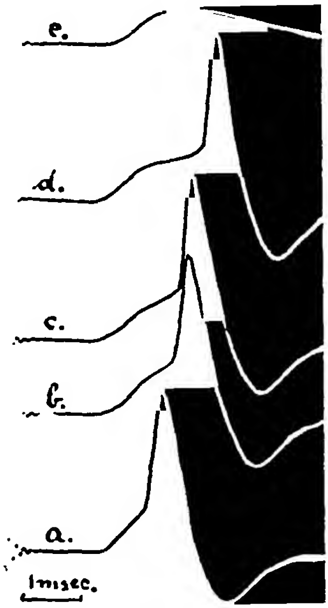

FIG. 6. Progressive curarization as in Fig. 5, some distance from the end-plate. a, before application of curarine; b, c, and d, show practically no change in e.p.p. height at the spike origin; e, pure e.p.p.

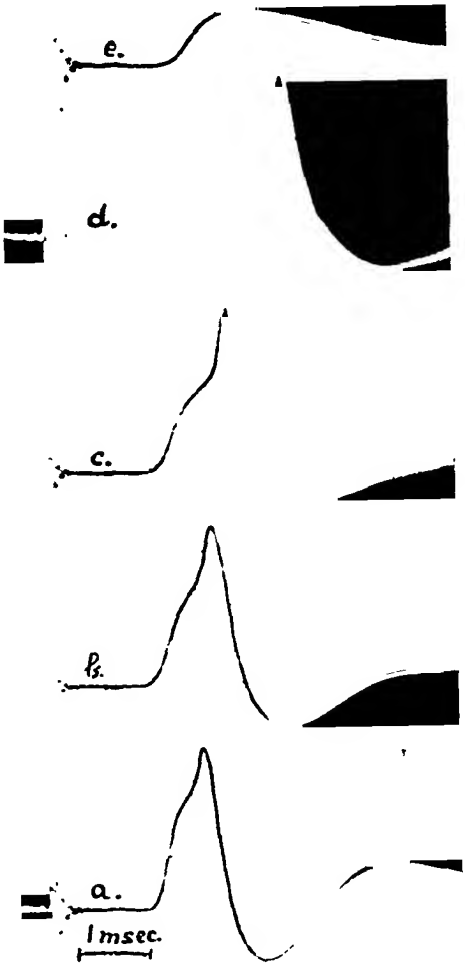

FIG. 5. Records taken at the end-plate region. a, before application of curarine; b, c, and d, during progressive curarization show the diminution of the initial end-plate potential (e.p.p.) and the progressive lengthening of the spike latent period; e, pure e.p.p., no spike is set up.

slowing of the conduction rate of the nerve impulse during curarization.

Figure 6 shows another set of observations during the application of curarine. The recording electrode was moved a little away from the endplate so that an initial e.p.p. of about 30 per cent total spike height was recorded. A striking difference from Fig. 5 can be seen in the curarine effect. The height of the e.p.p. at which the propagated impulse starts is not appreciably changed during progressive curarization, while all the other effects of cura-

rine are similar to those described above. Experiments of this type were done in six preparations in which the initial e.p.p.'s were normally only 20–30 per cent total potential height. The change observed during curarization never exceeded a few per cent.

DISCUSSION

It is at first surprising that the end-plate potential (e.p.p.) preceding the spike could not be found in the multifibre preparation of the isolated frog's sartorius (Eccles *et al.*, 1941a). Its occurrence in each single nerve-muscle fibre preparation is beyond doubt and the fact that these fibres gave consistent results for over 6–8 hr. while being frequently stimulated and manipulated suggests that they are in a good condition. However, these experiments have shown that very localized leading from the end-plate zone is necessary in order to detect the e.p.p. This does not seem possible in the whole muscle, where the aggregate potential from the scattered end plates would not show the sharp change in curvature which separates the e.p.p. and the spike in a single fibre.

Despite its close resemblance to the single fibre potentials, the double-step potential described by Schaefer and Haass (1939) could not have been due to an initial e.p.p. preceding the spike. Presumably it is produced by spikes in two different sets of muscle fibres as has already been suggested (Eccles *et al.*, 1941a). In the strip-preparations of cat's soleus a large initial e.p.p. (12–18 per cent peak-potential) was sometimes observed preceding the spike, but usually it was much smaller or even undetectable (cf. Eccles and Kuffler, 1941a).

There has been previous evidence (Eccles and O'Connor, 1939; Eccles *et al.*, 1941a; Schaefer and Haass, 1939) that the e.p.p. is responsible for initiation of the propagated spike when a subparalytic dose of curarine is applied, or during the refractory period. From the preceding experiments it is clear that the e.p.p. is also normally responsible for the initiation of the propagated muscle action potential. In most experiments an e.p.p. of 90 per cent total action potential height was found, and in many cases the e.p.p. formed the entire rising phase of the action potential at the end-plate (cf. Fig. 3e), the only sign of a propagated spike being the following diphasic part of the potential. It seems reasonable to suggest that e.p.p.'s at least as large as the spike potential are produced in all preparations but are not fully recorded on account of the relative smallness of the endplate and the difficulty of placing the electrode exactly on it. Moreover the end-plate does not reach round the whole circumference of the muscle fibre.

It would be of interest to know the threshold e.p.p. required for the initiation of a propagated impulse. Direct evidence might be obtained by recording at the exact position where the impulse starts; there the shortest spike-peak time would be obtained. For that a still finer method of leading would be necessary, but is not feasible at present. Further, the electrode shifts as a rule can be made in a longitudinal direction only, although rota-

tion of the fibre has been attempted. The absence of a spike component in Fig. 3e suggests that the spike arises some distance away from the point of recording. It would seem that the e.p.p. has to spread and depolarize the surrounding region sufficiently to set up a propagated impulse. This would be further supported if a point could be found near the end plate, where the spike starts whenever the e.p.p. reaches a given height. Indirect evidence on this point was obtained by the curarization experiments (cf. Fig. 6).

At the end plate (cf. Fig. 5d) the e.p.p. diminished to about 40 per cent potential height before the spike was abolished; the height of e.p.p. and spike initiation were apparently unrelated. At a little distance away, however (cf. Fig. 6), the spike seemed to take off from an approximately constant level of the e.p.p.; about 30 per cent of full spike height. The spike latency naturally increased with progressive curarization as a longer time was now required for the e.p.p. to reach the critical potential height. No spike appeared when the e.p.p. was slightly reduced (cf. Fig. 6d and e). This would suggest that the propagation along the muscle fibre starts whenever the region adjacent to the end plate is depolarized to about 30 per cent of the spike height.

From six experiments of this kind (as Fig. 6) it seems likely that the threshold level is about 30–35 per cent of the total action potential height.

The foregoing experiments show that the end plate itself has some different properties, regarding its excitability, from the rest of the muscle. A potential appreciably greater than necessary to initiate propagation at another point of the muscle fibre, can be set up without immediately propagating itself. Hodgkin's (1938) findings for nerve thus appear to obtain for the regions adjacent to the end plate, but not for the end plate itself.

As pointed out above, latency changes of e.p.p. rise during curarization were sometimes observed. These changes, if also occurring in cat, might explain the failure of Eccles and Kuffler (1941a) to obtain a similar time course of the e.p.p. and initial part of the spike in the "matching" tests, done on curarized preparations. Therefore the possibility of a quicker transmitting process than the e.p.p. could not be excluded in the cat experiments.

In the light of the present findings a still quicker mechanism for neuromuscular transmission need not be postulated for the single fibre preparation of the frog.

Summary

Single muscle fibres with their nerve supply have been isolated from the M. adductor longus of frog (*Hyla aurea*). Electric potential changes have been recorded at the nerve-muscle junction.

1. A potential of about the same size as the muscle spike potential is set up by the nerve impulse at the endplate, the end-plate-potential (e.p.p.).
2. This e.p.p. is responsible for the initiation of the propagated muscle impulses.
3. The e.p.p. can be greatly diminished by curarine before the spike is abolished.

4. There is evidence that the e.p.p. gives rise to the muscle spike by spreading electrotonically and critically depolarizing the region adjacent to the end-plate. It is suggested that the threshold for the initiation of a propagated impulse is about ⅓ of the normal e.p.p. height.

I wish to thank Dr. J. C. Eccles and Dr. B. Katz for their valuable help and assistance during the course of this investigation, and also the National Health and Medical Research Council of Australia for equipping and maintaining the workshop in which most of the apparatus was made.

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RESPONSES OF THE IRIS TO PROLONGED STIMULATION OF ITS PARASYMPATHETIC NERVE SUPPLY*

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ORIAS (1932) reported that stimulation of the preganglionic sympathetic fibers at relatively high frequency failed to elicit a sustained contraction of the nictitating membrane. When the postganglionic sympathetic fibers were stimulated under the same conditions the response of the membrane was maintained for long periods.

Lanari and Rosenblueth (1939) confirmed Orias' observation, and found in addition that prolonged continuous stimulation (2 to 3 hours) of the postganglionic sympathetic fibers resulted in a slow decline of the contraction of the nictitating membrane and of the dilator muscle of the iris. Stimulation of the preganglionic sympathetic fibers resulted in a more rapid decline of response (4th stage) followed by a late persistent increase (5th stage). A similar late increase of response took place when the vagal preganglionic parasympathetic nerves to the heart were stimulated. The 4th and 5th stages have also been observed during prolonged indirect stimulation of skeletal muscles (Rosenblueth and Luco, 1939).

The 4th and 5th stages appear, therefore, when sympathetic or parasympathetic preganglionic nerve fibers, or when motor nerve fibers are stimulated. All these nerves are cholinergic. The 4th and 5th stages, on the other hand, are not present during the stimulation of adrenergic postganglionic sympathetic fibers.

These data suggested the following questions. Is that difference in results due to the different nature—cholinergic or adrenergic—of the nerves? Or is it because in the first instance a ganglionic or a neuromuscular synapse is involved, while in the negative case the transmission occurs across a postganglionic smooth-muscle junction?

To answer these questions it was considered necessary to stimulate cholinergic postganglionic parasympathetic fibers and record the contraction of the smooth muscle innervated by them. The postganglionic fibers of the ciliary ganglion (short ciliary nerves) were selected, for three reasons: *a*, they are readily accessible; *b*, they are compact; and *c*, they form a relatively large bundle. The pupillary diameter was used as an indicator of contraction. The short ciliary nerves contain, however, some sympathetic fibers. It was necessary, therefore, to remove the superior cervical ganglion and wait for the degeneration of these fibers in order to test the effect of the parasympathetics alone.

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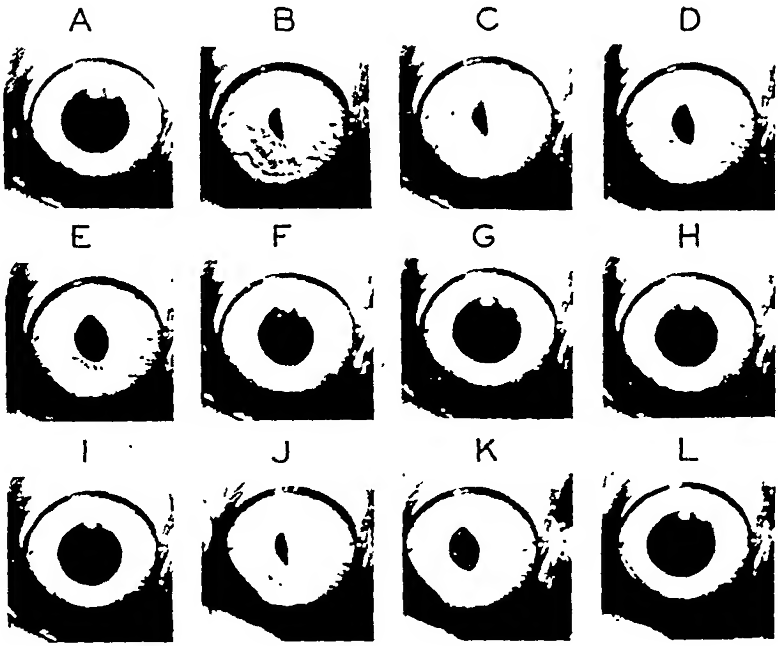

FIG. 1. Influence of frequency of stimulation of postganglionic parasympathetic fibers on responses of the iris. The superior cervical ganglion had been removed 10 days previously, to eliminate the sympathetic component of the short ciliary nerves. The stimuli were applied for one minute and the periods of stimulation were separated by 2-minute intervals. Photographs were taken 15 and 60 seconds after the beginning of stimulation; the pupillary diameter was practically the same at these times.

A, G and L are controls without stimulation. The other records are responses to different frequencies of stimulation as follows: B, 25; C, 50; D, 75; E, 100; F, 150; H, 200; I, 300; J, 25; and K, 17 per sec.

As a control for these observations some experiments were made in which the preganglionic parasympathetic fibers to the iris were stimulated in the IIId cranial nerve.

METHOD

Cats were used under dial anesthesia (Ciba 0.75 cc. per kg. intraperintoneally). To isolate the parasympathetic fibers it was necessary to remove a portion of the external wall of the orbit. Some of the external muscles of the eye were cut away and the ciliary nerves were dissected. The stimulating electrodes were placed beyond the location of the accessory ciliary ganglion which exists occasionally, lying distal to the ciliary ganglion (Whitteridge, 1937). When possible both the lateral and the medial short ciliary nerves were included within the electrodes. Often, however, it was not possible to use both branches. In those cases the main branch (lateral) was used alone (for the anatomy of this region see Whitteridge, 1937).

For stimulation of the preganglionic parasympathetic fibers in the IIId nerve the preparation was as follows. The external carotid on the unused side was ligated. The cervical sympathetic was cut bilaterally. After opening the skull a hemidecerebration was performed on the side which was to be stimulated. Electrodes were then placed on the exposed IIId nerve. Continuous suction by vacuum pump prevented the collection of cerebrospinal fluid or blood around the electrodes.

The pupillary changes were recorded either by measuring its transverse diameter or by photographing the eye at different times during stimulation. The stimuli were con-

denser discharges through a thyratron, controlled in rate by a frequency-beat oscillator. In a few cases a Harvard inductorium was used.

Results

A. *Stimulation of postganglionic fibers.* As explained under Method it was sometimes difficult to include all the short ciliary nerves in the electrodes. As a consequence the contractions of the pupil were not always uniform (Fig. 1 and 2).

In 12 animals series of brief periods of stimulation at different frequencies were recorded. Figure 1 illustrates typical results. It is interesting that the maximal construction was obtained at about 25 per sec., a frequency similar to those which yield maximal responses in other autonomic neuro-effector systems (see Rosenblueth, 1932).

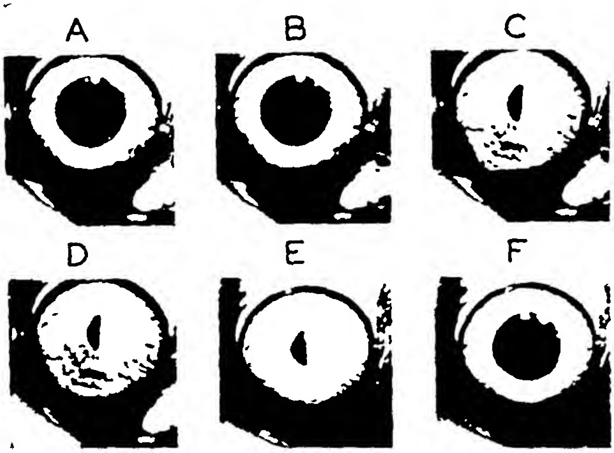

FIG. 2. Prolonged stimulation of the postganglionic parasympathetic supply to the iris at the frequency of 60 per sec. Superior cervical ganglion removed 12 days previously.

A and B: 2 and 0.5 min. before stimulation. C to E: 0.5, 2, and 120 min. after the beginning of stimulation. F, 1 min. after the end of stimulation.

For the study of the 4th and 5th stages the frequencies of stimulation used were 50 to 130 per sec. In 12 of the 20 experiments performed the frequency was 60 per sec., since Rosenblueth and Luco (1939) and Lanari and Rosenblueth (1939) found that this rate of stimulation was optimal for the separation of those stages.

Continuous maximal stimulation of the postganglionic parasympathetic fibers at the frequencies mentioned caused contractions of the sphincter muscle of the iris, which were maintained for 1 to 2 hours, as shown in Fig. 2. If the stimulation was prolonged further there was a gradual decrease of the response, but in no instance was there a late increase corresponding to a 5th stage.

In two experiments the postganglionic sympathetic fibers to the iris were stimulated for 1 hour at a frequency of 60 per sec. The results confirmed the report of Lanari and Rosenblueth (1939), *i.e.*, there was a dilatation of the pupil throughout the period of stimulation.

The responses to stimulation of either the sympathetic or the parasympathetic postganglionic supply to the iris are therefore similar. The contraction of the responding muscle is sustained for a long time. There is only a late and slight fatigue. There is no evidence of a late recovery (5th stage) following this fatigue (4th stage).

B. *Stimulation of preganglionic fibers.* Thirteen experiments were performed, with prolonged stimulation at frequencies of 25 to 150 per sec. With frequencies of 25 or 30 per sec. no fatigue was seen after a 90-min. stimulation. With frequencies of 60 to 150 per sec., typical 4th (Fig. 3C to F) followed by 5th stages (Fig. 3H to M) were recorded.

If, after the 5th stage had developed, the muscle was allowed to rest for

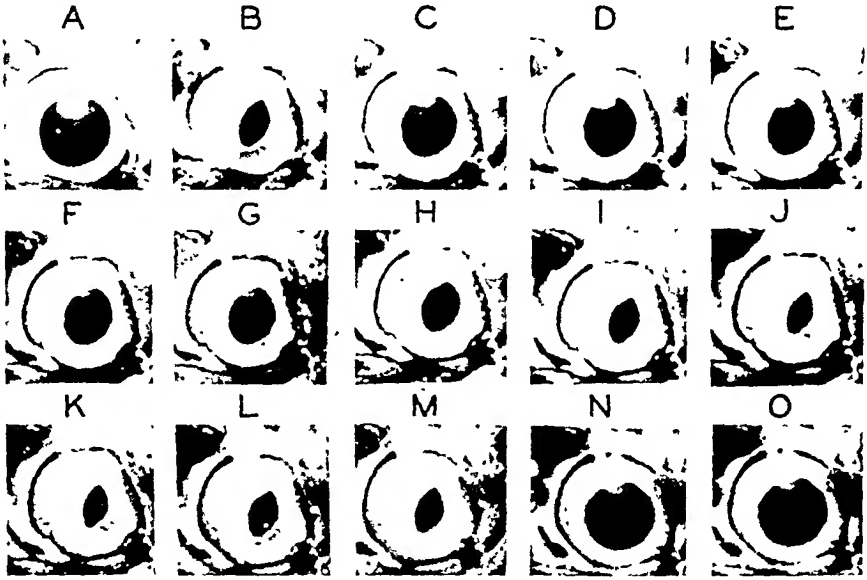

FIG. 3. Prolonged stimulation of the preganglionic parasympathetic fibers in the IIId cranial nerve at the frequency of 100 per sec. Adrenal glands ligated.

A: 2 min. before stimulation. B: 5 sec. after the beginning of stimulation. C to M: 2, 5, 15, 20, 26, 33, 40, 50, 64, 95 and 100 min. after the beginning of stimulation. N and O: 1 and 4 min. after the end of stimulation.

20 to 30 min., reapplication of the stimuli again ensued in a 4th and then in a 5th stage.

The results of stimulation of the parasympathetic preganglionic fibers to the iris are therefore similar to those found by Lanari and Rosenblueth (1939) in the preganglionic supply to the heart (parasympathetic) and to the nictitating membrane and iris (sympathetic).

DISCUSSION

That the parasympathetic component of the short ciliary nerves is cholinergic in the rabbit was shown by Engelhart (1931). In cats, similar results were obtained by Luco and Lissák (1938), *i.e.*, stimulation of these parasympathetic nerves releases acetylcholine in the aqueous humor of the eye.

During stimulation of the cholinergic postganglionic parasympathetic

fibers the typical results seen in other cholinergic fibers were not obtained (cf. Fig. 2 and 3). The results of that stimulation were similar to those obtained with adrenergic postganglionic fibers. It may be concluded, therefore, that the ability to elicit a late rise of response (5th stage) after an initial fall (4th stage) is not a general property of cholinergic nerve fibers, but only of preganglionic and motor axons.

Rosenblueth, Lissák and Lanari (1939) have shown that the five stages of ganglionic or neuromuscular transmission may be explained by changes in the output of acetylcholine from the presynaptic nerve endings. If this explanation is accepted, it should be assumed that the output of acetylcholine from postganglionic parasympathetic fibers does not undergo the changes which take place in preganglionic or in motor nerve fibers. The output of acetylcholine in postganglionic elements declines slowly during a prolonged stimulation. Similarly, the release of adrenaline from adrenergic fibers probably decreases only slightly over prolonged periods of stimulation.

Summary

The optimum frequency for brief periods of stimulation of the parasympathetic nerve supply to the cat's iris is 25 per sec. (Fig. 1).

Prolonged stimulation of the postganglionic parasympathetic fibers at frequencies of 50 to 150 per sec. results in a contraction of the sphincter of the iris which is sustained for 1 to 2 hours and which thereafter gradually declines (Fig. 2). There is no evidence of a 4th stage followed by a 5th stage of increased contraction.

Similar prolonged stimulation of the preganglionic fibers in the IIId cranial nerve results in a marked contraction followed by a fall (4th stage) and later by an increase of response (5th stage).

It is concluded that the appearance of the 5th stage is not a property of all cholinergic nerves, but only of preganglionic and motor fibers.

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ISOLATION OF RETINAL AND OPTIC GANGLION RESPONSE IN THE EYE OF *DYTISCUS*

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Introduction

It has long been recognized that the electroretinogram of the vertebrate eye in the standard leads "cornea-back of the bulb" consists of several component potentials. Algebraical summation of such potentials has been held to account for the polyphasic response to illumination (Kuehne and Steiner, 1880; Einthoven and Jolly, 1908; Piper, 1911; Kohlrausch, 1918). The presence of three such components, PI, PII, PIII has been established by the experiments of Granit and his collaborators (see summary by Granit, 1938).

One of the difficulties with the vertebrate eye is that localization of the components has to be carried out on an eye in which the receptors are directly joined to two ganglionic layers. For this reason it is of importance to carry out some analytical work with other types of eyes in which different layers of the visual apparatus are anatomically separated or separable. In the eye of the water-beetle I have found it possible to separate the visual cells from the optic ganglion and also to demonstrate how electrotonic spread may complicate evaluation of a complex potential made up of the primary visual response and the electrical phenomena in the ganglion. The outcome of this work will be reported below.

Actually, the eye of *Dytiscus* has been studied previously by Adrian (1936) who did not attempt to discriminate between potentials from different sources but chiefly devoted himself to describing various types of rhythmic activity recorded from the optic ganglion. Hartline in 1925 gave a brief description of electroretinograms from different insect eyes without attempting to analyze them. Later Jahn and Crescitelli (1938, 1939; see also Crescitelli and Jahn; 1939a, b), have recorded the complex response to light of a number of insects. Probably the simplest visual response hitherto recorded is that of the arthropod *Limulus polyphemus* (Hartline, 1925; Hartline and Graham, 1932) which is wholly due to the receptors. Recent work by Therman (1940) indicates that even a single stratum of visual cells in the eye of cephalopods may react to light in a relatively complex manner (*cf.* old work by Beck, 1899; Piper, 1904; Fröhlich, 1914). The comparison with the results of other workers will be found in the discussion.

COMPOUND EYE AND OPTIC GANGLION OF *DYTISCUS*

The compound eye of the water-beetle occupies a large part of the lateral wall of its head and, according to Leinemann (1904), consists of 9.000 ommatidia placed fan-like. To each ommatidium belongs a cornea-lens and the sexangular cornea facettes form a highly convex surface. The distal curvature of each lens (cl in Fig. 1) is insignificant but the proximal surface is highly curved so that the compound cornea is of considerable thickness.

According to the work of Guenther (1912) the *ommatidium* (Fig. 1) is built in the following manner: Just inside the *lens* there are 4 Semper's cells (kkzk) surrounding a conical *crystal interior* part (kk). These form a cover around the crystal cone reaching to the distal parts of the outer retinula cells (kks). The *retinula cells* (rt) are a drawn-out cell. group with a distal swelling in the region of the nuclei of the outer cells (rtzk) and a proximal enlargening caused by the nuclei of the basal retinula cells (bz). In the prototype ommatidium there are 7 radially placed retinula cells around an eighth central cell. This type of ommatidium with 8 visual cells seems to be present in the majority of insects and should be regarded as the original form (Plate, 1924). But in the water-beetle (Guenther, (1912) as well as in many other beetles (Plate, 1924) the central cell descends down towards the basal membrane to form the so-called basal cell (bz). Of the remaining radially placed cells one is expelled in the proximal zone and therefore does not take part in the formation of the rhabdome.

The *rhabdome* (rh) is a rod-like homogeneous structure surrounded by the retinula cells and placed in the centre of the ommatidium. It consists of a highly refractive substance, probably formed by the inner surfaces of the six radial cells and the basal cell. From the rhabdome excitation is held to be transmitted to the retinula cells (visual cells).

From the latter and the basal cell the nerve *fibres* arise (nf), and penetrate the basal membrane soon to end in the optic ganglion. According to Guenther they pass a layer of nerve cells just under the basal membrane. According to Holste (1923) this layer would merely be a dendritic branching of the fibres. Between them would be supporting cells and pigment cells.

The optic *lobe* is a 1.5 mm. long pear-shaped structure with its basis towards the compound eye and the apex tailing off into an optic nerve. Its organization is very complex. The general plan seems to be that the nerve cells are found in an outer layer surrounding a central nucleus of interconnected dendrites (for details, see Holste, 1923). From the optic lobe the optic nerve runs to the supra-oesophageal ganglion (sg Fig. 2). The distance from the layer of retinula cells to this ganglion is about 2.5 mm.

In the eye of *Dytiscus* the dioptric apparatus of each ommatidium is at some distance from the rhabdome (see Fig. 1). At moderate intensities the various ommatidia are optically badly isolated on account of the lack of pigment between them. In this state the eye of *Dytiscus* should be regarded as a *superposition eye.* This means that rays from the object reach the same rhabdome through several facettes there to add their excitatory effects. Intense illumination, however, leads to movement of the pigment in between the ommatidia. This favors optical isolation. Thus a smaller number of rays from a given object are projected onto each rhabdome. The decreased summation leads to decreased sensitivity and the eye changes into an *apposition eye* (Plate, 1924).

The pigment cells (ip) bringing about this change are partly found distally round the crystal cone, and partly at the basis of the retinula cells. Intense illumination makes the pigment of the outer layer move inwards towards the basal membrane, whereas the inner pigment moves towards the cornea. Below a certain strength of illumination follows withdrawal of the pigment.

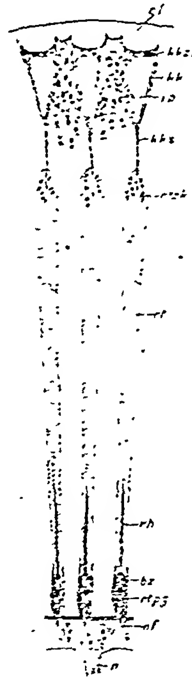

FIG. 1. Section along three ommatidia in the eye of the water-beetle. Explanation in text (after Guenther, *Z. wiss. Zool.*, 1912, *100:* 60–115).

TECHNIQUE AND PROCEDURE

Apparatus. Recording of the action potentials was carried out with a cathode ray oscillograph in conjunction with a three-stage push-pull directly coupled amplifier, designed by the physicist of this laboratory, Mr. T. Helme. Absence of drift has been systematically controlled. Time was recorded in 0.2 and 0.4 sec. with a Rayleigh-wheel. As stimulus served "white light" from a hundred watt bulb; Wratten neutral tint filters of standardized intensity were used for variations of intensity.

Preparation. (Cf. Adrian, 1937.) The wall of the isolated head was lifted up and the underlying strong muscles removed until the supra-oesophageal ganglion and the optic ganglion were layed bare. These ganglions were freed from adjoining tissue, and the supra-oesophageal ganglion was lifted up to rest upon an electrode (5 in Fig. 2) taking care to keep the optic nerve between them intact. In this manner the optic nerve and the optic ganglion were isolated by air up to the basal membrane, and the other electrode could be given different positions on the optic pathway. In order to lead off from the front of the facette eye a small hole was cut in the chitinous cornea through which the fine point of the electrode could be inserted. Owing to thickness of the lens this could be done without damaging the visual cells.

As electrodes were used silver-chloride pins covered with cotton wicks drawn out into a thin point. Now and then the preparation was irrigated with Ringer solution. Leads could also be taken from the isolated compound eye after cautious removal of the optic ganglion (leads 1–2). In such cases histological control showed that the point of severance was in the region of the basal membrane.

When dark-adapted animals were used these had been in the dark for 12 hours. As pointed out by Adrian (1937) the optic nerve may keep alive for 3–4 hours and the ganglion for a very much longer time if prohibited from drying. In quantitative work 2 hours has been the limit in these experiments and the repeated response to a standard intensity has been used as a check upon the state of the preparation.

Results

Intact preparation. Record a of Fig. 3 shows the typical effect from the optic ganglion (leads 3–5 of Fig. 2) of a fresh preparation and is in essential agreement with pictures given by Adrian (1937). As pointed out by him the electrode (5) on the supra-oesophageal ganglion can be regarded as a reference electrode. This is shown, for instance, by the fact that crushing this ganglion does not alter the potential change. The baseline before the onset of light in Fig. 3 shows that there was no definite resting activity. Illumination leads to a strong sustained negativity under the electrode of the optic ganglion relative to the proximal electrode. (In this as in all other records an upward deflection marks negativity of the distal electrode relative to the proximal one.) The negativity of the ganglion first rises rapidly, then more slowly to a plateau. If illumination be continued for a longer time the potential very slowly diminishes (cf. Fig. 4a and 5a). On the slow potential (Fig. 3a) there are superimposed irregular oscillations, some of the nature of spikes, others slower changes. These stop abruptly at cessation of illumination and after an upward peak the negativity diminishes. This rather complicated picture recurs with great regularity when the active electrode is placed on the proximal part of the pear-shaped optic ganglion.

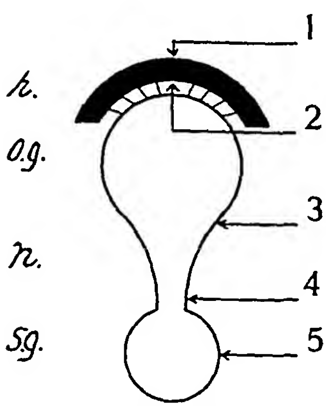

FIG. 2. Schematic drawing of an eye prepared for an experiment with receptorial layer (r), optic ganglion (og), optic nerve (n), and supra-oesophageal ganglion (sg). The numbers 1–4 show the different loci used for the active electrode, whereas 5 is the constant locus of the reference electrode on the supra-oesophageal ganglion.

Some of the typical "later reactions" (Adrian) of this preparations are

illustrated by Fig. 3b, c and d. After an hour, and sooner if the preparation is damaged or drying, the irregular oscillations of low amplitude, caused by strong illumination, tend to fall into a well developed regular thythm at 10–20 beats per sec. This "bright rhythm" follows upon illumination (see Fig. 3b and 4b) and disappears when the light is cut off. On the other hand, if the preparation be left in the dark there arises a rhythmic discharge ("dark rhythm") with a frequency of 6–8 per sec. (Fig. 3d), which disappears upon illumination to be replaced by the "bright rhythm." When the light is turned out the "dark rhythm" returns progressively, as illustrated by Fig. 3c. Adrian came to the conclusion that these rhythms were to be regarded

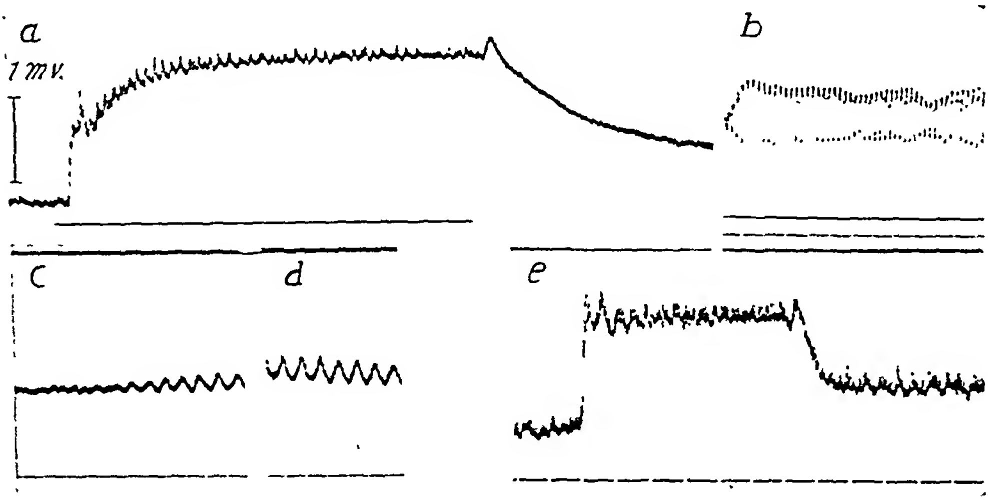

FIG. 3. a, potential activity of the optic ganglion (leads 3–5) following illumination of the eye. b, "bright rhythm." c, beginning of the "dark rhythm." d, "dark rhythm" fully developed. e, as in a, but with spontaneous activity present. Time in ⅕ sec.

as the result of synchronized activity in a large number of units. Strong illumination or darkness seemed to be the main conditions for the appearance of synchronized beats. In the former case the rhythm was held to reproduce the maximal frequency of the neurons, in the latter their natural resting discharge.

Figure 3e shows a special phenomenon visible also in records 5a and b. In this case the ganglion has begun by discharging spontaneously, chiefly rapid spikes, in the dark. Upon illumination there is added to these the fast potentials caused by the light. At cessation of illumination the latter disappear, as in Fig. 3a, at the same time as the upward "off-deflection" appears. But, whereas in Fig. 3a the baseline after cessation of illumination is quiet, just as it was before the eye was illuminated, in Fig. 3e there recurs on the falling phase of negativity the spontaneous activity noted already in the dark. The interesting point here is that the spontaneous activity is inhibited during the "off-deflection" itself, the latter being almost free from spikes.

The farther up on the optic nerve the active electrode is placed the smaller the negativity caused by illumination. Records a and b in Fig. 4, with the active electrode placed between 3 and 4 (Fig. 2) have been taken with a faster film. Here spike activity is better visible on the slow potential. There is a heavy initial discharge slowly falling to a constant frequency kept up as long as illumination sustains the negative potential (cf. Fig. 13). Similar leads have been used for record 5a and b after development of the

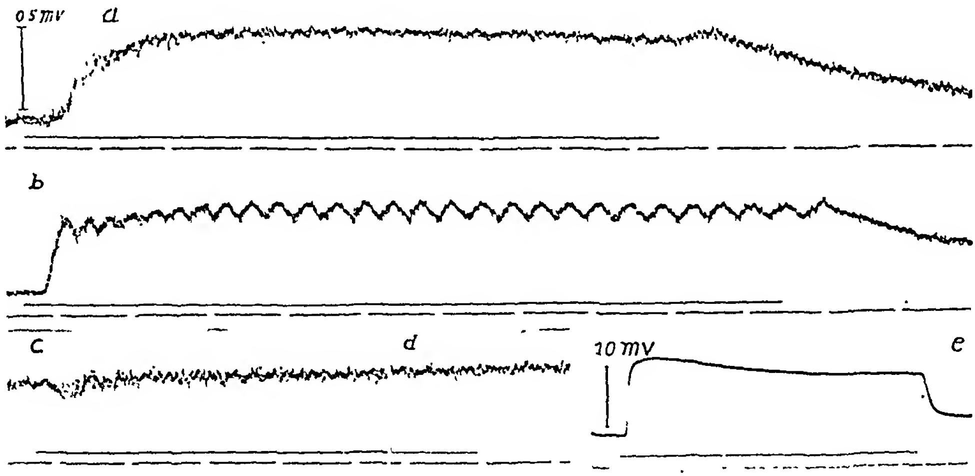

FIG. 4. a and b, action potentials from the optic ganglion in the fresh (a) and ageing (b) preparation. Active electrode between 3 and 4. c and d, spike potentials from the optic nerve (leads 4–5) at "on" (c) and "off" (d). e, potential of isolated compound eye (leads 1–2). Time in 1/5 sec.

regular "bright rhythm." In this figure should be noted the rhythmic grouping of the rapid spike potentials. In parallel with the development of the slow beats at 12 per sec. the spike potentials are found to be grouped chiefly on the rising phase of the potential wavelets. As long as these are absent, as in record 4a, the spike potentials are relatively evenly distributed (cf. Adrian, 1937).

In leads 4–5 on the nerve in the vicinity of the s.o.-gangilon (Fig. 4c) there is no slow potential whatever but only spike potentials in the nerve arranged in groups corresponding to the rhythm of record 4b. The downward swing of these spike potentials in records 4a and b suggests that in this case they come from the proximal electrode. Such pictures are sometimes obtained and signify that the electrode on the s.o.-ganglion is placed near the point of entrance of the optic nerve. The off-deflection is clearly visible in records 4a and b and these as well as Fig. 4d demonstrate the abrupt cessation of spike potentials when the light is turned off.

This series of pictures thus shows how the typical effect, as led off from the optic ganglion, consists of a slow negativity which diminishes in ampli-

tude when the active lead wanders towards the s.o.-ganglion. Cessation of illumination is followed by a definite off-deflection accompanied by inhibition of spike activity. Rhythmic waves may occur on the slow negativity and their frequency after illumination seems to coincide with the frequency of the grouped impulses in the nerve.

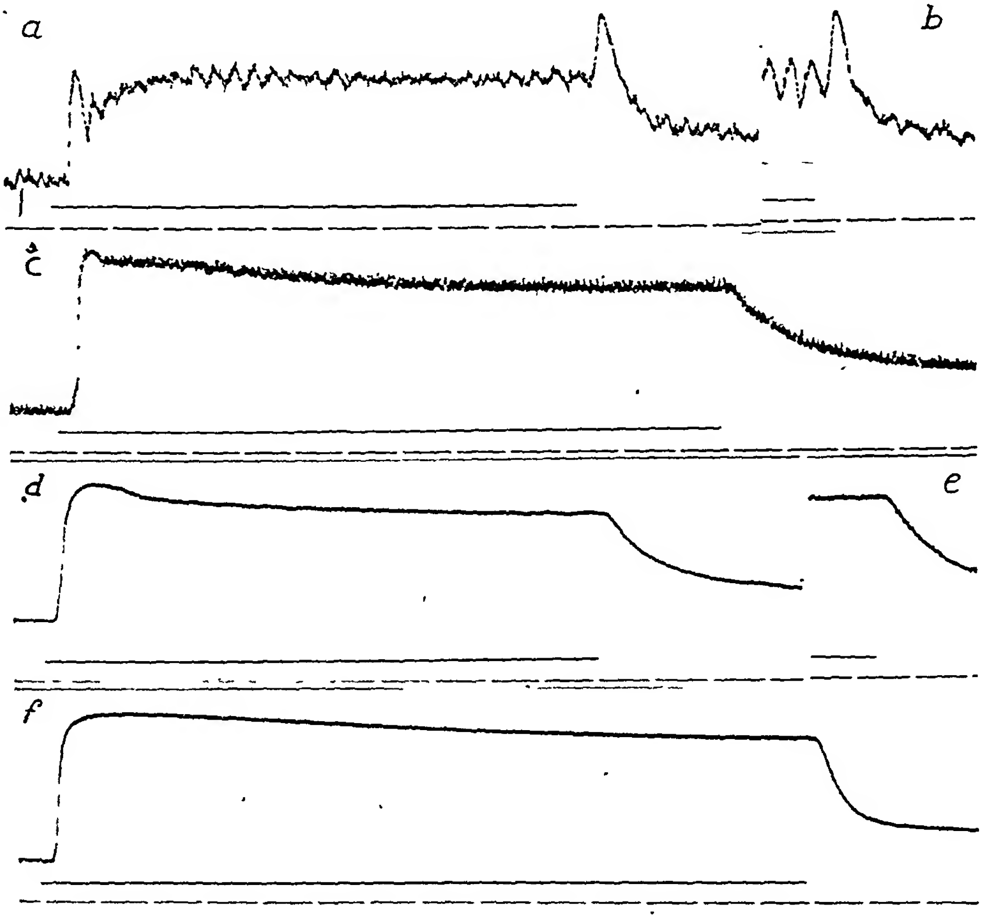

FIG. 5. Cocaine experiments, a and g, normal optic ganglion potential (leads 3–5). c, some minutes after 4 per cent cocaine. d and e, later after cocaine. e, potential of isolated compound eye control (leads 1–2). Time in $\frac{1}{5}$ sec.

If the optic ganglion and the central parts of the visual path be severed from the compound eye in the region of the basal membrane and leads 1–2 be used, illumination still elicits a large slow potential of upwards 10–15 mV as shown by Fig. 4e. This record is from a moderately dark-adapted eye. The electrode in the front, without exception, is negative relative to the electrode on the back of the eye. In this case the negativity directly rises to

its maximum, and after a drawn-out top passes over into a slightly sloping plateau (cf. Fig. 9a). When the light is turned out the negativity first drops quickly to a certain level from which follows a very slow descent towards the baseline. This is best seen in slow films, as Fig. 9a. The isolated compound eye (*i.e.* the retina *minus* optic ganglion) always gives a monophasic effect. There are never any superimposed potential waves nor negative off-deflections.

This circumstance that the isolated compound eye only gives a strong negative potential upon illumination suggested that the sustained negativity, led off from the optic ganglion, in reality belongs to the eye itself. Experiments with cocainized ganglions, as we shall see, support this conclusion.

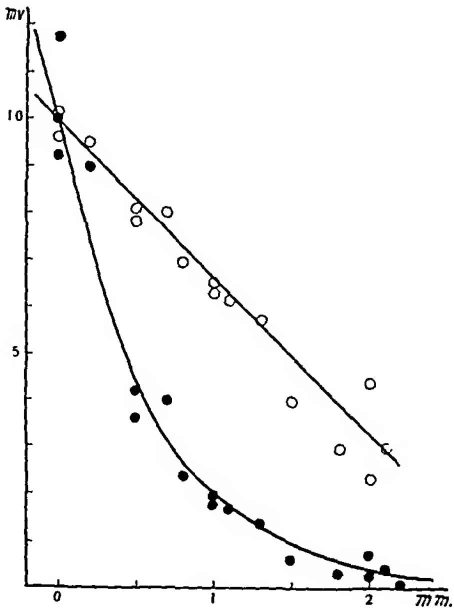

FIG. 6. Decrement of the retinal action potential along the visual path plotted in terms of size of response against distance in mm. from the receptors (filled circles). The log of these values (open circles) also plotted against the same abscissa. Zero on the abscissa represents the potential as led off directly from the compound eye itself.

Ganglionic action potential after cocainization. In record a in Fig. 5 there is first the characteristic response from the optic ganglion (leads 2–5) already described. There is definite spontaneous activity in the dark and a tendency to rhythmic waves before and after illumination. After the initial rise of the potential there is a definite swing-back before the effect slowly rises towards its maximum (cf. Fig. 3a). When a drop of a 4 per cent cocain solution has been applied to the ganglion the type of the response totally changes (Fig. 5c). The electrodes have not been moved. The initial swingback has disappeared, and so have the rhythmic potential waves as well as the off-deflection. There remains only the large negative potential upon which is superimposed an even flow of impulses in which transition from spontaneous activity to stimulated activity cannot be discerned. At a later stage spike activity also disappears and the negative potential of Fig. 5d tends to assume the shape of the isolated retinal action potential from the compound eye alone (Fig. 5f, leads 1–2). Even an increase of the type of exposure (Fig. 5e) cannot provoke an off-deflection (cf. Fig. 5b).

This and other similar experiments demonstrate that the effect, as recorded from the optic ganglion, partly consists of a strong sustained negativity almost certainly emanating from the receptors, and partly of effects

from ganglion and nerve fibres superimposed upon the latter. The optic ganglion contributes an effect both at "on" and "off" in addition to potential waves during illumination. These effects sum algebraically with the potentials from the retina. The relation between the retinal action potential and the ganglionic effect will be discussed below.

Decremental spread of the retinal response along the optic pathway. The experiments with cocain made it highly probable that the response remaining after cocaine is nothing but the retinal response picked up from the ganglion. Seeing how in Fig. 4 the amplitude of the sustained negative response diminishes, the further the leads are from the receptorial layer, it became necessary to investigate this factor systematically with our standard illumination and electrode 5 on the s.o.-ganglion as reference point.

Four such experiments have been averaged in the diagram of Fig. 6 to show the amplitude of the response (filled circles) against distance of the active electrode from the receptorial layer. Zero on the abscissa means that the leads are taken from the compound eye itself (1–5 in Fig. 2). Size of the visual response falls along the optic pathway in an exponential manner, as shown by the straight line obtained when log amplitude is plotted against distance (open circles in Fig. 6).

The spatial decrement of the slow negativity in the diagram suggests electrotonic spread. The decrement follows the equation $P_p = P_r \cdot e^{-ax}$ in which P_p represents the maximal amplitude at the postretinal electrode at distance $\times$ cm. from the receptorial layer and P_r is the maximal amplitude below the receptor electrode (cf. Bogue and Rosenberg, 1934 for polarization of peripheral nerve and Eccles, 1935 for the post-ganglionic trunk in the superior cervical ganglion). The electrotonic constant a in this case is about 16, indicating relatively limited spread of the retinal action potential.

A polarizing current of sub-threshold strength and rectangular shape gives in the sciatic nerve of frog at zero distance an electrotonic potential with approximately exponential rise (Bogue and Rosenberg, 1934). With increased distance of active electrode from the polarizing circuit the electrotonic potential has an S-shaped rise and a lower and somewhat later maximum. Similar changes with increased distance from the point of origin also characterize the sustained negativity of the eye of the water-beetle. Thus, record 4a which has been taken at a distance of about 1.5 mm. from the eye illustrates the slow creep from the baseline and S-shaped rise of the response. Even when taken directly from the compound eye the potential does not leave the baseline instantaneously, although it rises far more rapidly and sooner reaches its maximum compared with the preceding case (visible, for instance, in Fig. 4a compared with 5f despite different speed of the film).

The slow rise of the potential makes it difficult to locate its earliest point. Another complication is the on-effect from the ganglion, mentioned above (see Fig. 3a and 5a), which falls into different regions of the rising phase dependent upon the locus of the active electrode. But taken as a whole these experiments on electrotonic spread support the conclusion that the

main part of the sustained response is nothing but the retinal action potential which has spread electrotonically to the ganglion.

The potential of the compound eye. One may ask whether the monophasic, relatively simple retinal action potential is homogeneous or whether it consists of components with different function or significance. From this point of view the striking difference between its rising initial phase and its descent to the baseline is of particular interest. Apparently illumination leads to a depolarization of the receptorial layer causing its proximal end to be negative relative to the neurones.

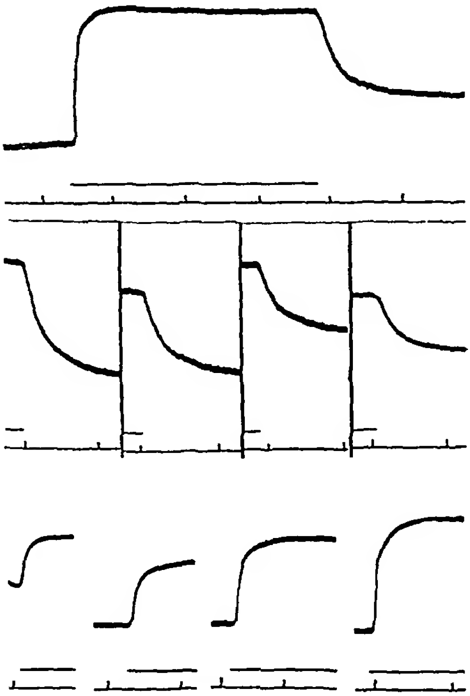

FIG. 7. Potentials of isolated compound eye (leads 1–2). Upper record, control. In the middle row off-effects after respectively 0.5, 1, 15, and 30 sec. illumination. Lower records illustrate "on"-effects for intervals 0.5, 4, 15, and 30 sec. after cessation of previous illumination. Time in 1/5 and 1/1 sec.

In Fig. 4a and 5f, and particularly after the long exposure in Fig. 9a, one can distinguish two phases with different temporal properties in the fall of the curve after cessation of illumination. There is an initial rapid drop, varying in extent from case to case, and falling to a level from which the potential then slowly diminishes. The first rapid phase is complete within about 0.2 sec. But it lasts some 30–60 sec. before the potential has reached zero from this level. These changes are clearly dependent upon time of exposure.

A. Different times of exposure. Records in the middle row of Fig. 7 show the fall of the retinal action potential of the isolated compound eye for different times of exposure (0.5–30 sec.): Between each exposure there has been an interval of rest of more than 1 min. and the maximal amplitude of the negativity has been checked up and found to be constant and of the order of magnitude of the initial deflection in the upper record of Fig. 7. After an exposure of 0.5 sec. the retinal response drops rapidly about 85 per cent of its initial value to be followed by the slowly diminishing remainder. But after an exposure of 30 sec. the fast drop of the response is only about 40 per cent of its initial rise. At the same time it tends to tail off more slowly into the later phase. An experiment of this type is shown schematically in Fig. 8, where the course of the fall of the response towards the baseline for different exposures is put in along the sustained negativity and the values are given in per cent of the maximal ampli-

tude of the negativity. The dotted curve shows the level reached by the rapidly falling phase at different exposures.

As already pointed out the initial rapid drop lasts about 0.2 sec, but it takes up to a minute before the potential almost rectilinearly reaches zero. If after a previous exposure the stimulus is reintroduced, the light causes reappearance of the visual response which then reaches an amount of potential which roughly corresponds to the level attained by the preceding retinal action potential. And the new effect rises from that particular point

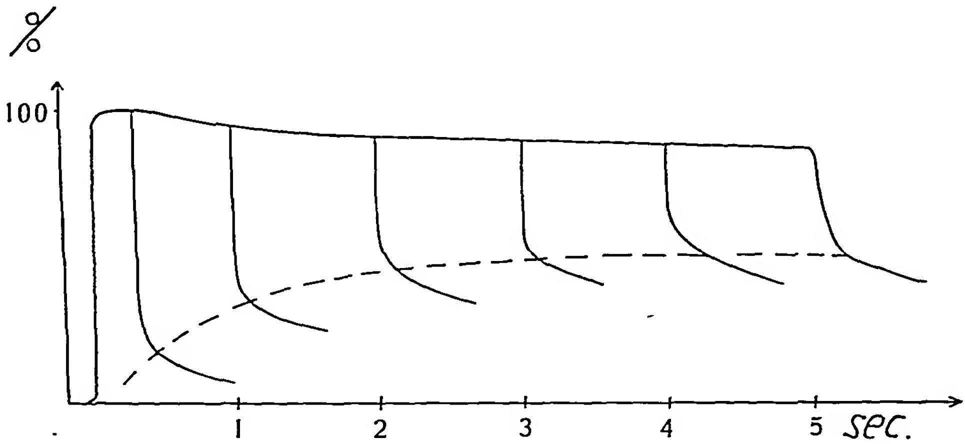

FIG. 8. Diagram showing the course of fall of the retinal negativity towards the baseline after varying times of exposure. The dotted line shows the level to which the rapidly falling phase of the vanishing response drops at "off." The values are in per cent of the maximal amplitude of the retinal response.

on the slowly falling phase of the preceding stimulus which corresponds to the interval between cessation of the one and initiation of the other response. Thus, on account of the fall in the level of the potential, as it slowly declines in the dark, the amplitude of the reintroduced visual response will increase when this interval of darkness increases. A series of such responses recurring after different intervals is shown in the lowermost record of Fig. 7 to be compared with the uppermost curve of the same figure taken after a dark interval of more than a minute.

The upper record of Fig. 9 shows the sustained visual response of the isolated compound eye after an exposure of 90 sec. The potential slowly falls during the first 30 sec. thereafter to remain at a practically constant height. The lower record shows the effect of a very slow flicker at 1–2 per sec. With this intermittent stimulus the upper contour is a copy of the effect of continuous illumination. The lower contour marks approximately the level of the rapid drop, as with this rate of stimulation the slow fall has not time to appear before the next flash of the intermittent stimulus sets in. Compared with the experiment, shown in Fig. 8, the level to which the rapid drop of

potential falls, sooner reaches its maximal value which also is greater than in Fig. 8. The determinants for these variations will be discussed below.

B. Different intensities. In the experiments illustrated in Fig. 10 a long exposure of 20 sec. has been chosen so that the rapid fall at "off" is small

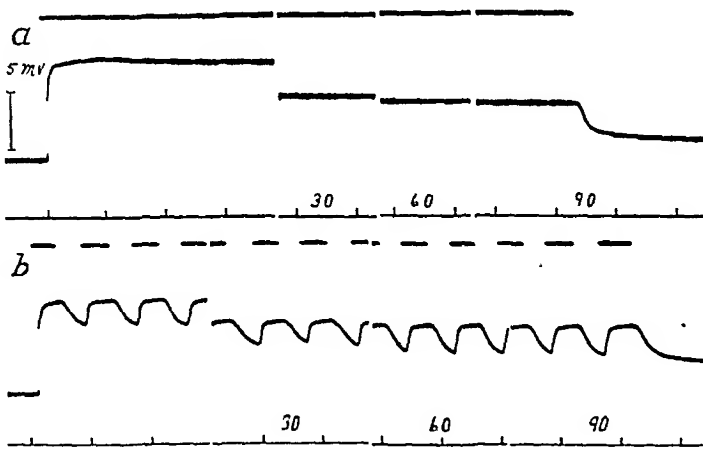

FIG. 9. Response of isolated compound eye to 90 sec. of continuous (a) and intermittent (b) illumination. Time in sec.

(cf. Fig. 8) at the highest intensity (intensity 1 in Fig. 10a). The initial drop at "off" is only about 10 per cent of the maximal amplitude of the response.

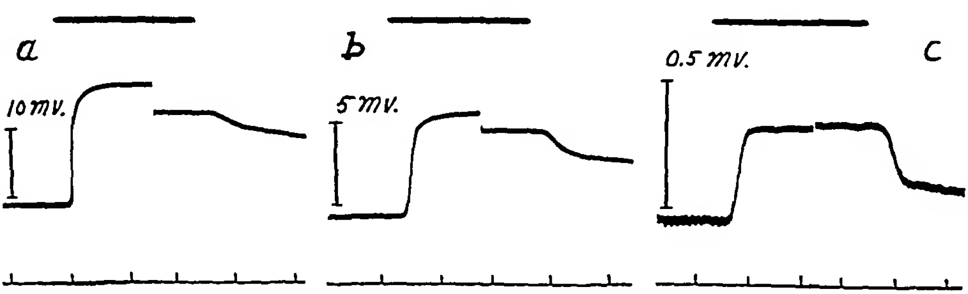

FIG. 10. Response of isolated compound eye to 20 sec. illumination with intensities 1 (a), 1/100 (b) and 1/10,000 (c). Time in sec.

When illumination was diminished to 1/100 the total retinal action potential decreased to less than half, whereas the initial drop at "off" was 20 per cent and thus in absolute terms was practically unaltered. Finally, at the intensity 1/10,000 the retinal response was reduced to 1/50 and now the rapid fall practically reached the baseline. The average result of 5 such experiments is shown graphically in Fig. 11. In all experiments time of exposure has been 20 sec. and 5 intensities have been tested. Both the values for the

total amplitude of the retinal action potential (filled circles) and the rapid drop at "off" (open circles) are plotted in per cent of the maximal response against log intensity. The diagram shows how the retinal action potential

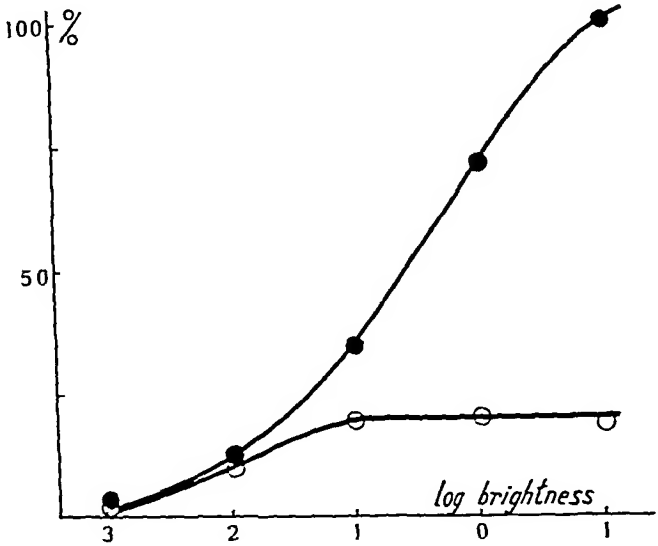

FIG. 11. Plot of the amplitude of the total response (filled circles) and of the rapid drop at "off" (open circles) against log brightness. Values in per cent of total response to maximal intensity (intensity 1).

rises as the intensity increases. The amplitude of the rapid drop at "off," however, follows quite a different course. It is clear that, as long as the curves run together, this also means that the whole visual response disappears with the rapid drop at "off." This is seen to be the case at the lowest intensities (1/10,000, 1/1000). But at the higher intensities from 1/100 upwards the rapid drop of potential at "off" asymptotically reaches a maximum although the total visual response now increases very rapidly. In proportion to the latter the fall therefore looks smaller and smaller. Thus, at higher intensities, an increasingly greater part of the total fall of potential at "off" will consist of the slowly falling phase. The rapid depolarization at "on" and the rapid repolarization at "off" behave very differently at different intensities.

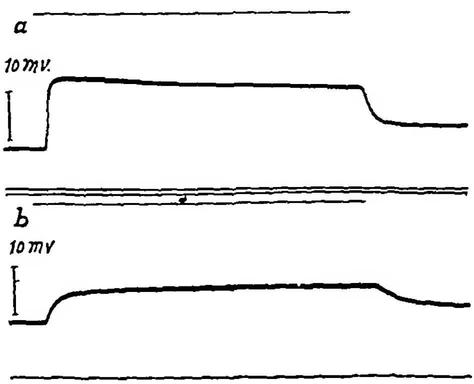

FIG. 12. Response of dark-adapted (a) and light-adapted (b) isolated compound eye. Time in ⅕ sec.

Figure 12 shows the difference between the retinal action potentials of light- and dark-adapted water-beetles. After prolonged dark-adaptation

the visual response rapidly reaches large values and also disappears rapidly when the light is turned off. But if the eye is left illuminated for 8–10 min. and then, after 1 min. in the dark, is stimulated with the standard intensity (lower record in Fig. 11) the visual response looks quite different. Its rising phase is slower and amplitude lower and at cessation of illumination it also disappears along a drawn-out curve.

Discussion

Adrian (1937) held the large potential recorded from the optic ganglion of the water-beetle including the rhythmic beats in darkness and those elicited by bright light to be due to the optic ganglion itself. But we have seen now that the retina of the isolated compound eye lacking its ganglion cells reacts to light with a large sustained negativity spreading electrotonically along the optic path down to the ganglion from which it also can be recorded. Even if Guenther's view that there is a separate layer of cells just below the basal membrane be correct, these cells can hardly be responsible for this very large potential, the less so as histological controls have shown that in some of the experiments with isolated eyes even the basal membrane has been torn away. Illumination clearly leads to a depolarization of the receptors themselves making their distal end negative in relation to the back of the eye including the post-receptorial fibres.

The eye of cephalopods (Beck, 1899; Piper, 1904; Fröhlich, 1913) which also consists of receptors alone gives a similar monophasic negative potential. Among arthropods the lateral eye of the horse-shoe crab (*Limulus polyphemus*) is known to react in the same manner (Hartline, 1928; Hartline and Graham, 1932). The eyes of other arthropods (*Melanoplus chortophaga*, *Vanessa*, *Musca*, *Bombus*) according to Hartline (1928) may show a diphasic variation of the chief negative potential. Later work with insects (*Melanoplus samea*) has shown that sometimes a positive initial deflection may precede the prolonged negative receptor potential (Crescitelli and Jahn, 1939a, b; Jahn and Crescitelli, 1938–39). But these experiments have been carried out on eyes *in situ*. Under such circumstances complex responses may be obtained also from the retina of *Dytiscus*, which when properly isolated always respond with a simple monophasic potential.

It is obvious that polyphasic responses may be caused by currents spreading over inactive tissue to the second electrode which cannot be regarded as indifferent (cf. Gilson and Bishop, 1937; Eccles, 1939). Complex responses may also be caused by processes in the optic ganglion as shown above. Thus, for instance, Hartline's picture from *Vanessa* looks like the combined response of retina and optic ganglion in *Dytiscus* (leads 1–5).

These considerations do not exclude the possibility that in the receptors of some insects there may be processes of opposite potential sign. They merely serve to emphasize that such phenomena have not yet been convincingly demonstrated. Actually Therman (1940) has found two processes of opposite potential sign in the isolated eye of the cephalopod *Loligo*, re-

acting somewhat differently to different agents. Whether similar phenomena can be shown to occur in the eye of the water-beetle remains to be investigated. So far they have not turned up.

On the other hand it will be necessary to deal with two monophasic potentials of identical sign in *Dytiscus*, as shown by the experiments of Fig. 7, 8, 10 and 11, analyzed above. The response of the *isolated* compound eye to relatively strong light is shown split into its two components in Fig. 13. The dotted line S of Fig. 13a represents the level to which the total

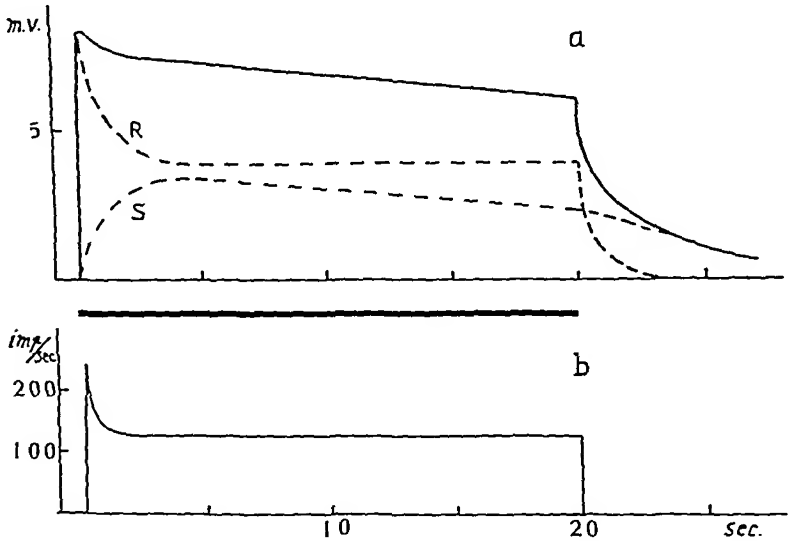

FIG. 13. a, analysis of the electroretinogram of the isolated compound eye (line drawn in full). Components drawn in broken lines, heavy black line indicates a stimulus of a duration of 20 sec. b, frequency of the impulses in the optic nerve plotted against duration of illumination.

potential falls upon cessation of illumination and is taken to illustrate the course of the slow component. It rises so slowly that at short exposures the total response has a large rapid drop at "off." After long exposures the drop at "off" decreases owing to the rise of S. As indicated in connection with Fig. 12 the latter component is largely responsible for the shape of the retinogram of the light-adapted eye. The difference between the total response and the superimposed slow component is given by curve R in Fig. 13a, illustrating a second fast component the peak of which probably is unduly accentuated. This component seems to be better marked in the dark-adapted eye and looks like an integrated frequency-time curve of the impulses in the optic nerve (see Fig. 13b). As shown by the experiments of Fig. 10 and 11 the maximal amplitude of the total response rises when the stimulus is being increased, whereas the fast phase of the repolarization at "off" rises only inside a relatively low range of intensities. At a certain level of intensity the initial deflection goes on increasing with further increases in

strength of illumination but the drop at "off" remains constant. This implies that the total response at low intensities chiefly consists of the fast component R whereas at higher intensities S becomes relatively more prominent. This explains why at low intensities (see Fig. 10c) the record shows an instantaneous drop at "off" and lacks the rounded top of the high intensity responses. There is further to be noted that at low intensities (Fig. 10c) the response remains at constant height whereas at height intensities it slowly decreases during illumination (see Fig. 10a and b). Unfortunately the peak of the component R cannot be obtained experimentally. This is because R predominates only at low intensities and because the peak probably is absent with weak stimuli, to be judged from the fact that the peak also is absent in the frequency-time curve of the impulses at such intensities.

Can we make any assumptions as to the nature of these components? Hanström (1937, 1938) distinguishes between two systems: Peripheral retinula cells with short nerve connections (*fibrae visuales breves*) to a first synapse, and basal retinula cells with relatively long nervous connections (*fibrae visuales longae*) to a more centrally placed layer of ganglions in the optic lobe. These suggestions have to be borne in mind as providing a possible explanation of the two component potentials in the isolated compound eye of *Dytiscus*, even though another explanation at the moment seems to be more probable. This is based on the fact that at higher intensities the retinal pigment moves up between the ommatidia. The slow course of the S-component is in favour of this view, as is also the fact that the impulses come and go much as the fast component R. In the dark-adapted state and at low intensities the pigment is contracted; it expands as the intensity rises and then again it slowly withdraws upon cessation of illumination. These properties do remind one of the properties of the slow component.

A comparison with the vertebrate retina suggests that the slow component might be identical with PI responsible for the c-wave (Granit, 1933–38; Granit and Riddell, 1934; Therman, 1938). The component PIII (Granit, 1933–38; Therman, 1938; Granit and Helme, 1939) is the one most likely to be the fast component R of the water-beetle's eye. But, taking into account the inversion of the vertebrate retina (Kuehne and Steiner, 1881), they are of opposite sign, and for this reason previous workers (Bruecke and Garten, 1907; Piper, 1911) have been inclined to identify the negative response of the cephalopod eye with the b-wave of the vertebrate retinogram which is part of its component PII (Granit, 1933). A possible solution of this discrepancy may be found in Terman's (1940) result that the receptor potential of *Loligo* has two components of opposite sign, both with properties reminiscent of the vertebrate component PIII. On this view PIII may turn up with different electrical sign, predominantly positive, predominantly negative, in different types of eye and under different experimental conditions.

Summary

Action potentials have been recorded from the isolated compound eye, the optic ganglion, and the optic nerve of the water-beetle (*Dytiscus marginalis*) with a directly coupled amplifier and a cathode-ray oscillograph.

The aim of the work has been to separate the activity of the isolated retina from the potentials in ganglion of nerve in order to study the properties of the former, all of which have been mixed in previous work with the same preparation.

The receptors in the isolated compound eye upon illumination react with a large monophasic potential, the front of the visual elements being negative relative to the back of the eye and postreceptorial fibres. No potential of opposite sign has ever been seen.

The properties of the retinal response have been studied in experiments with varying exposures and intervals of rest at constant intensity as well as for varying intensities at constant exposures. The experiments suggest that the monophasic response consists of two components of the same electrical sign: A slow one somehow connected with adaptation, and a fast one representing receptor activity. In the dark-adapted state and at low intensities the fast component predominates; in the light-adapted state and with strong stimuli the low component becomes visible.

The retinal response spreads to the optic ganglion with electrotonic decrement. The effect, led off from the optic ganglion, consists of the retinal response adding itself to specific potentials from the optic ganglion. There are in the latter deflections at both "on" and "off," and, under certain circumstances, rhythmic wavelets during illumination. The specific potentials in the ganglion disappears, when it is cocainized, so that finally only the monophasic response from the retina is left. The rise of the receptor potential coincides with an outburst of impulses in the optic nerve, soon falling to a constant frequency during illumination. When the light is turned off the impulses stop instantaneously and there is never any off-discharge to be seen.

During the off-deflection in the ganglion spontaneous activity in the nerve, if present, is inhibited.

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THE ACOUSTIC AREA OF THE MONKEY (*MACACA MULATTA*)

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THE TRANSVERSE temporal gyrus, Heschl's convolution, has traditionally been regarded as the site of termination of the acoustic thalamo-cortical radiations in man. Two principal lines of evidence may be adduced in support of this belief. Cytoarchitectonic and myelogenetic studies point to this area as one of characteristic histological structure (area 41 of Brodmann). Clinical studies, though meagre, tend to show that damage to the transverse temporal gyrus may result in hearing deficiencies.

In the monkey, there is usually on the superior (horizontal) surface of the superior temporal gyrus, near its broadened posterior extremity, a slight elevation which, it has been suggested, may be homologous with the human transverse temporal gyrus. Poliak (1932) states that the largest contingent of fibers of the acoustic radiation of the monkey terminates in the cortex of the elevation, although he also describes radiation fibers passing to all concealed surfaces of the superior temporal gyrus. Walker (1937), using the same species, found that a portion of the elevation is occupied by an area of koniocortex which he assumed to be primary acoustic projection area. Lesions involving this part of the superior temporal gyrus resulted in retrograde chromatolysis in the medial geniculate body.

The present study is an attempt to demonstrate by an electrical method the exact limits of the primary cortical acoustic projections, as has previously been done on the cat (Ades, 1941).

METHOD

In order to obtain adequate exposure of the superior temporal gyrus, it is necessary to remove the cortex and medullary substance of the inferior parietal and frontal lobes as illustrated in Fig. 1A. The entire hemisphere is exposed under deep nembutal anaesthesia and the areas mentioned are removed by means of a suction pipette so that the inferior lip of the lateral fissure as well as the insula is cleared, affording access for the electrode. The entire experiment is carried out under nembutal anaesthesia.

The stimulus consists of a sharp click generated by a thyratron relaxation-oscillator working through a power amplifier and delivered from a 10-in. speaker 2 ft. from the animal's head. The oscillator could be fired manually or by a timer. Recording of the stimulus was accomplished by means of a microphone placed close to the animal's head, and led through a transformer-coupled amplifier to the oscillograph.

Response from the cortex was recorded by leading from a unipolar silver electrode through a resistance-capacity coupled amplifier to a Dumont type 175A oscillograph.

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Simultaneous recording of stimulus and response on the same cathode-ray tube was accomplished by splitting the beam with a Dumont electronic switch.

Standard procedure was to move the electrode systematically in steps of approximately 2 mm. over the exposed inferior lip of the lateral fissure, recording the response from each point until the responsive area had been delimited. The responses shown in the figures were photographed from the single sweep (manually controlled) cathode-ray trace on 35 mm. Eastman 809 recording paper.

Results

Click stimulation produces an essentially diphasic response in the primary acoustic projection area of the monkey. The response is characterized by an initial large surface-positive spike, followed by a surface-negative phase, usually smaller in magnitude and longer in duration. Typical examples are shown in Fig. 2. It has essentially the same characteristics as the cortical response to appropriate stimulation in other afferent systems, as described by several previous authors. Some variability in the response is encountered. For example, at times the surface-positive phase seems to be the only component present. This is frequently due to the masking effect which the spontaneous potentials exert on the negative wave, as is shown by the fact that the negative component is nearly always perceptible when the base line is smooth. At times, the negative component is the larger or even the only visible portion of the response, a variation which may occur at any point within the boundaries of the area. This particular variation is unsystematic in its occurrence, and, from the results of the present study, can only be interpreted as due to temporary local conditions at the time recorded. The criterion for determining the boundaries of the acoustic projection area was, therefore, essentially

Fig. 1A. Fronto-lateral view of monkey brain showing exposure of superior temporal gyrus and insula.

Fig. 1B. Lateral view of monkey brain; black lines indicate extent of acoustic area as projected to lateral surface.

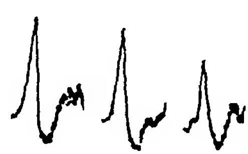

Fig. 2. Photographs showing typical cortical response to click stimulation.

presence or absence of response, since the variability in the response was unsystematic, even erratic.

The portion of the superior temporal gyrus of the monkey responsive to click stimulation was confined to the posterior half of the superior surface, adjacent to the insula. The area usually covers the medial half of the supe-

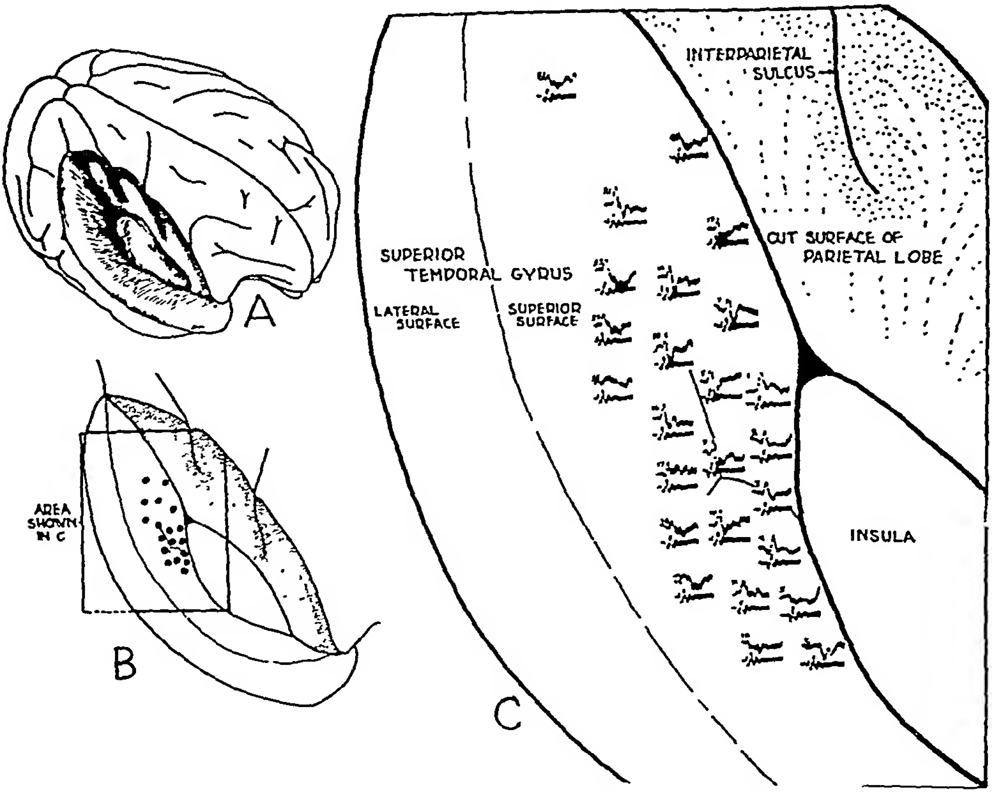

FIG. 3. Monkey No. 11, right side. A. Fronto-lateral view of brain, showing experimental exposure. B. Enlarged view of exposure; black dots indicate points from which responses were obtained. C. Enlarged view of area outlined in B, showing photographs of responses superimposed at point from which each was elicited. Lower trace in each case is the stimulus line.

rior surface, sometimes more, but never extends as far laterally as the exposed lateral surface of the gyrus.

Maximal responses were always from the angle formed by the posterior and medial margins of the superior surface, that is, from the medial end of the elevation referred to by Poliak, when the elevation is present. Responses diminish somewhat in magnitude as the electrode is moved farther from the angle, but the margin of the responsive area is marked by disappearance of response within a short distance. The gradient from center of responsive area to periphery is, therefore, not gradual, and from periphery to non-responsive cortex occurs an abrupt dropping-off of response. This is illus-

trated by Fig. 3 and 4, which show, on diagrams of the superior temporal gyrus of 2 monkeys, the responses at various points inside and outside of the area. In several instances it may be seen that adjacent points (approximately 2 mm. apart) give, at one point, marked response. and at the other, no response.

The area of responsive cortex is quite constant from one monkey to an-

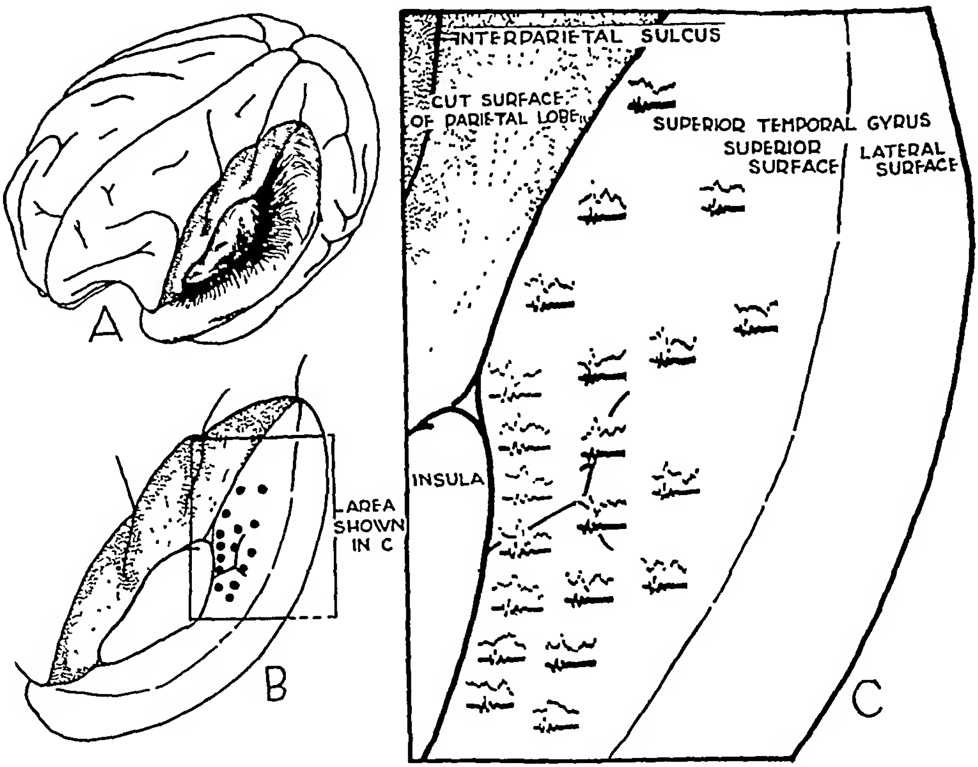

FIG. 4. Monkey No. 12, left side A. Fronto-lateral view of brain, showing experimental exposure. B. Enlarged view of exposure, black dots indicate points from which responses were obtained. C. Enlarged view of area outlined in B, showing photographs of responses superimposed at point from which each was elicited. Lower trace in each case is the stimulus line.

other, as may be seen in Fig. 5. The angle of the medial and posterior margins, at the posterior extremity of the insula, which is the widest point on the superior surface, is always the approximate geographic center. The remainder of the area extends in either direction along the two margins for varying distances. It seems probable that if it were possible to place an electrode on the medial (vertical) surface of the superior temporal gyrus, one would also obtain responses there, since it is evident in most cases that the responses reach maximum at the medial border of the superior surface,

where the cortex turns to form the inferior vertical wall of the Sylvian fossa, opposite the inferior half of the insula.

No responses were elicited from the anterior half or the lateral-most strip of the superior surface of the superior temporal gyrus or from its lateral surface. The posterior extremity of the superior surface was also invariably

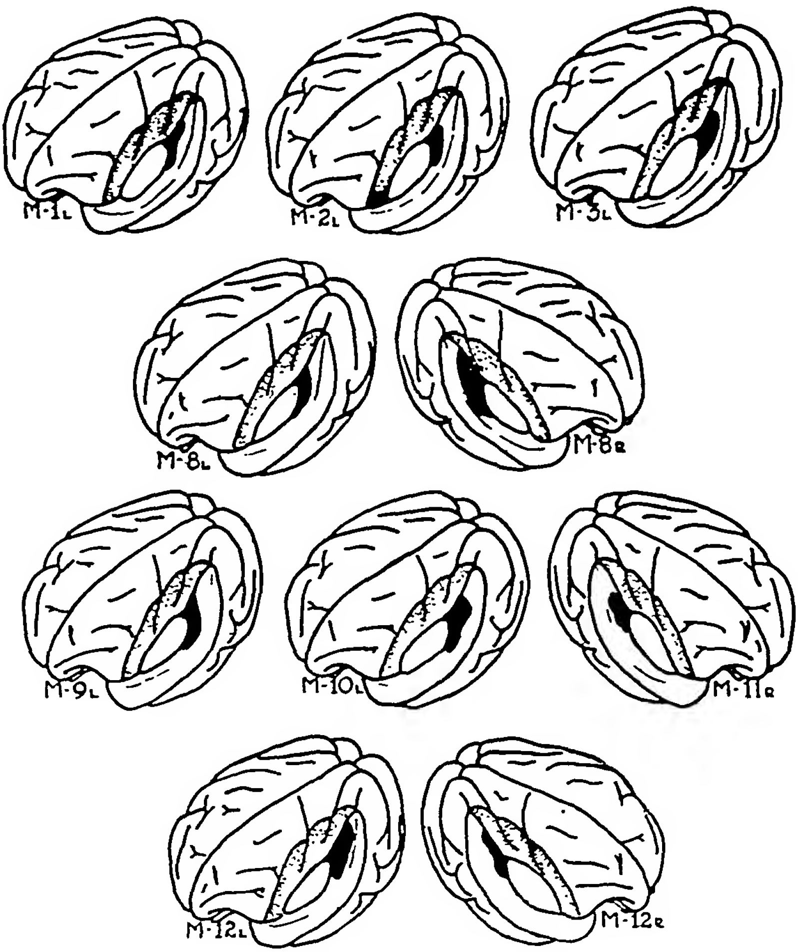

FIG. 5. Black-shaded portions indicate extent of responsive cortex in each case. Numbers indicate serial number of animal; small letters indicate right or left side.

silent to acoustic stimulation. This is apparently at variance with the anatomical findings of Poliak who described much more extensive endings of acoustic radiations, even to the lateral surface of the gyrus, and particularly to the posterior extremity. It is not easy to account for this apparent discrepancy. If it were possible to demonstrate a gradually diminishing magnitude of electrical response from a central maximum to a peripheral minimum, it would be easy to correlate magnitude of response with density

of innervation, since apparently Poliak's results indicate such a gradient of density of innervation. There is, to be sure, a border of less than maximally responding points around the periphery of the projection area, but the difference in magnitude of response between that border and the center of the area is not great, and two millimeters beyond the border the response drops out abruptly.

Walker states that the small temporal area, which he found to have the characteristics of koniocortex, "... is situated on a small elevation to which Poliak has previously called attention, and suggested that it might be a rudimentary transverse temporal gyrus." In the present study when the elevation is present, the medial half, which occupies the angle referred to above, is always responsive to click stimulation, whereas the lateral half is not. Furthermore, the responsive area nearly always overlaps the elevation both posteriorly and anteriorly. Thus, while part of the elevation is acoustic, part is not; on the other hand, not all of the acoustic responsive area is to be found on the elevation. These facts might indicate doubt that Poliak's elevation is truly a rudimentary transverse temporal gyrus. The question is of little importance, but it should be borne in mind that there is insufficient evidence to support the usual idea that the acoustic projection area is conterminous with Heschl's convolution. Similar experiments on the human brain might well show that there is a similar lack of absolute coincidence of transverse elevation and responsiveness to acoustic stimulation. There is no reason, therefore, to oppose Poliak's suggestion of homology between the transverse elevation of the monkey and the human transverse temporal gyrus.

Summary

By the method of recording cortical response to click stimulation, the acoustic cortex of the monkey has been mapped. The area responsive to clicks is the portion of the superior face of the superior temporal gyrus included in the angle between the posterior and medial borders of the gyrus. It varies in extent from 8 to 12 mm., and is consistent from one animal to another.

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THE BREAKDOWN OF ACCOMMODATION—NERVE AS MODEL SENSE-ORGAN

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IN EVERY highly developed sense-organ it is possible to distinguish a primary recipient mechanism, adjusted for maximal sensitivity to the adequate stimulus, and a secondary "generator" mechanism firing the nerve or perhaps ganglion cells directly. The very special problem as to how different forms of energy are translated into a physiological stimulus capable of exciting the generator mechanism will not be considered in this paper. We shall only be dealing with the nature of this secondary mechanism, a problem belonging to the general physiology of the special senses.

At present it is only possible to attack this general problem with the aid of hypotheses to be tested experimentally. The hypothesis to be chosen in the first instance is one which is suggested by the relatively extensive work on the retina. From this work it is known that the simpler the retina or the better the isolation of components of the response of complex retinae, the more definite the evidence for the conclusion that the generator mechanism is a slowly rising potential preceding excitation or inhibition, as the case may be (see Adrian and Matthews, 1928, Hartline and Graham, 1932, Hartline, 1938, Granit, 1933, 1938, Granit and Therman, 1935, Bernhard, 1941). There is enough evidence for the reasonableness of this general assumption to be found in the exciting properties of "local" potentials in nerve (Hodgkin, 1938), muscular end-plates (Göpfert and Schaefer, 1936), Eccles, Katz and Kuffler, 1941, Eccles and Kuffler, 1941), salivary glands (Langenskiöld, 1941), spinal neurons (Umrath, 1933, Barron and Matthews, 1938) to make the earlier results with the retina in no way exceptional. From the point of view of neurons and sense-organs, however, the main question is how repetitive firing is brought about by this potential difference. Thus "accommodation" enters the problem.

The work on accommodation shows that a non-accommodative nerve reacts to a rising current with a stream of impulses (Fessard, 1936, Erlanger and Blair, 1936, 1938) much as an adequately stimulated sense-organ would do, and that any nerve can be made to acquire such properties *e.g.* by suitable treatment with citrate or Ca-free Ringer (Solandt, 1935–36, Katz, 1936, Lehmann 1937, Schriever and Cebulla, 1938). It then tends to fire spontaneously. The same holds good for ganglions (Bronk, 1939) and sense-organs (Matthews, 1931 for muscle-spindle, Talaat, 1933 for sense-organs in the skin), many of which discharge spontaneously without having been subject to treatment of any kind. We have found (unpublished work) the retina also to increase its normal spontaneous activity after treatment with citrate-Ringer.

If sense-organs set up repetitive discharges as a consequence of "local"

potentials arising in them, then the question of *breakdown* of accommodation in nerve in response to slowly rising currents deserves a separate study. Beyond the general knowledge that a strong catelectrotonus causes a nerve to fire repetitively there is very little information concerning repetitive firing in normal vertebrate nerve. Such use of nerve as model sense-organ should show to what an extent imitation of the "generator" potential imitates some of the known properties of sensory discharges. Thus, for instance, the retina has a long latent period, 10–20 times any possible synaptic delay. It can react with excitation or inhibition, gives grouped or otherwise synchronized discharges, etc.

METHOD

For slowly rising currents of different time constants an apparatus built by our physicist, Mr. T. Helme, was available. The principle of this instrument is a release of an initially blocked anode current by charging the grid over condensers. Kahlson and v. Werz (1936) among others have utilized this principle before. An improvement in our apparatus consists in the use of a suitable resistance connected to the cathode-side of the H.T. circuit in order to eliminate deformation of the H.T. current rise owing to the characteristic of the valve. The form of the rise of the current in the stimulating anode circuit was determined oscillographically. In the diagrams plotted below, however, the actual rising times have not been used but a factor proportional to the C×R products of the condensers charging the grid. The reason for this was our original intention to calculate Hill's constant λ (Hill, 1936, and below). The stimulating currents remain at the level to which they have risen until broken off manually. A description of the technique together with observations on the breakdown of accommodation has been given in Swedish by Granit and Skoglund (1941).

Silver-silver chloride electrodes placed in the anode circuit were held to be satisfactory on account of the high internal resistance of the H.T. circuit relative to that of the tissue. The nerves were stimulated monopolarly through these electrodes. A Lapicque-circuit similar to the one used by Solandt (1935–36) for slowly rising stimuli was also available.

Decerebrate cats, sometimes frogs, were used for work on peripheral nerve. In the former case the animals were kept in a heated and shielded box with a cistern of boiling water supplying the moist atmosphere needed. A number of experiments were also carried out with the retina and optic ganglion of *Dytiscus*.

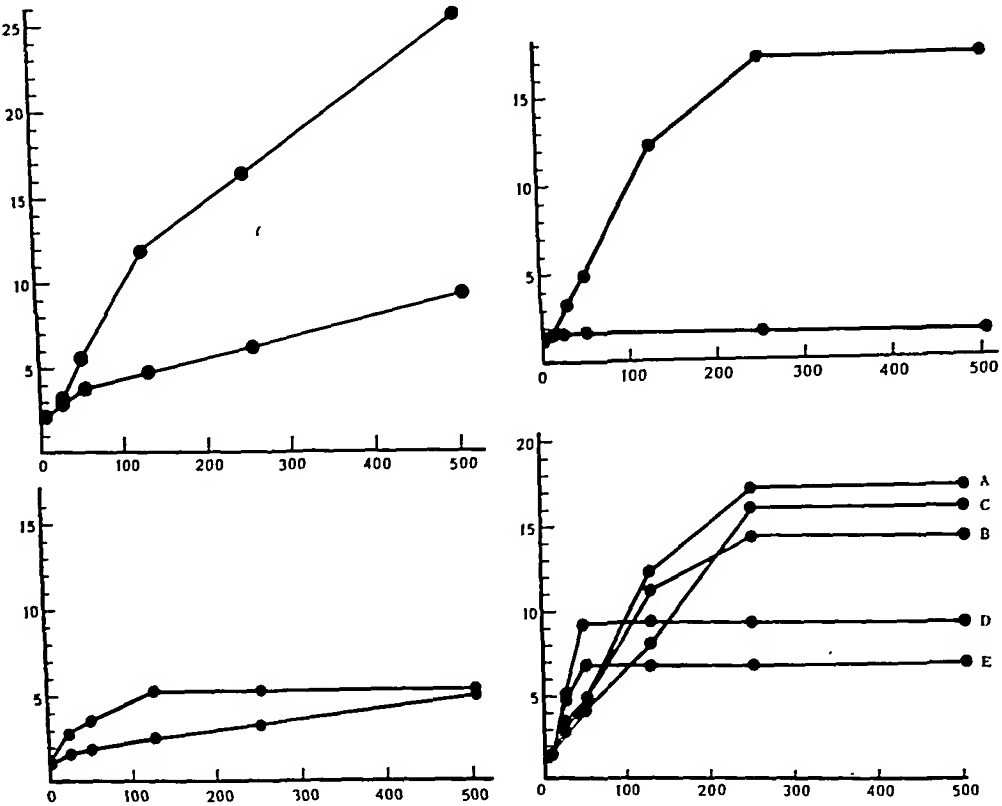
Cathode ray oscillograph and condenser coupled amplifier were used for recording from nerve or muscle.

RESULTS

1. *The index for accommodation measurements*

In 1884 it was shown by von Kries that, as the rate of rise of the stimulus decreased, the currents had to be made stronger in order to excite. He also plotted curves showing current strength as ordinates against rising time as abscissae. Following Hill's (1936) theory Solandt (1935–36) and Katz (1936) plot their results in the same manner but using multiples of rheobasic strength as ordinates and a factor proportional to rising times as abscissae. As a consequence Hill's accommodation constant λ appears as the inverse value of the slope of these curves which for the short rising times in which they were interested were rectilinear. Thus, with little accommodation, λ is great, the curves approach horizontality, and the nerves even tend to fire repetitively (Katz, 1936). Index in these measurements was a threshold muscle contraction.

In actual practice these measurements may be made in several different ways and we also began by using a threshold muscle contraction as index and determined the current strength necessary for a given rate of rise. Such experiments showed that what we have called "breakdown" of accommodation can be demonstrated already by observing the curves obtained in this



FIGS. 1–4. "Accommodation curves" for mammalian nerves with threshold muscle contraction as index. Abscissae: Rising time of stimulus in msec. (see text). Ordinates: Multiples of rheobase. Rheobase unit = 1 in all curves, but curves not always continued to this unit. *Fig. 1:* upper curve transected sciatic, lower curve transected peroneal nerve. *Fig. 2:* upper curve, unsevered peroneal nerve, lower curve, *n. aurient. magn. Fig. 3:* A–C: unsevered peroneal nerve, A immediately, B after 4 min., C after 25 min. D–E: same nerve severed and electrodes near cut end. *Fig. 4:* severed sciatic: upper curve electrode about 4–5 cm. from cut end, lower curve at cut end.

manner. Some curves selected from a large number of experiments are given below.

Figure 1 shows two curves for the sciatic and the peroneal nerve, both severed, Fig. 2, two intact nerves, *n. peroneus* and *n. auricularis magnus*, in the latter case with a reflex muscle contraction (pinna reflex) as index. The auricular nerve generally gave curves of this type. In Fig. 3 the three upper curves (A, B, C) were taken in this order during half an hour with the elec-

trode on an intact peroneal nerve. Then the nerve was severed and the electrodes placed near the cut surface. The curves D and E were obtained. The results are typical. Finally, in Fig. 4, a sciatic nerve, sectioned from the beginning, was used, but at a relatively late stage, the lower curve showing the result of putting the stimulating electrode near the cut end of the nerve.

According to Hill (1936) and his collaborators, whose interest was restricted to the early part of these curves, the inverse value of the slope is a measure of accommodation. Thus, the more the curves approach horizontality, the less the capacity of the nerve to accommodate. At the outset we shall define complete breakdown of accommodation by taking from the diagrams the strength of current at which the curves turn round to become horizontal. Beyond this point the nerve responds to any current above the strength of this ordinate independently of its rate of rise. In the sample curves of Figs. 1–4 breakdown occurs with different degrees of completeness and at different strengths in terms of multiples of rheobase.

A glance at these curves shows that accommodation in mammalian nerves is extremely sensitive to interference with the tissue (cf. also Schriever 1932, Liesse, 1938, a, b for blood supply etc. and frog nerve.) But there must also be unknown physiological factors to account for variations which certainly are not caused by maltreatment of the nerves. It seemed worth while investigating whether such variations had something to do with the observation that the muscle contraction was an unsatisfactory index. For rapidly rising currents the threshold contraction was brief and precise, but with slowly rising stimuli it also became slow, looking then more like a contracture. Hoffmann in 1910 had already found that the action currents then indicated repetitive firing. Schriever and Cebulla (1938) have repeated and confirmed these observations using frog's nerve which apparently in the normal state accommodates much better than mammalian nerves.

In repeating this work with leads to the amplifier from the stimulated nerve we found that the index for accommodation was equally uncertain in this case. There is no doubt repetitive firing from the nerve (cf. also Rosenblueth, 1941), developed more with slowly rising stimuli, but the question as to when and how the breakdown of accommodation takes place, is still unsolved, except that it is clear that, as predicted by Hill's theory (1936), in a general manner there is less accommodation when the slope is smaller. But it is obvious that the difficulty of deciding whether a brief or a protracted muscular contraction should be used as the constant index, necessary for obtaining the accommodation curves, is by no means solved by exchanging it for the difficulty of deciding what kind of nervous discharge to use as index, since the latter also is far from being constant but varies with respect to duration, number of spikes and amount of potential. For whole nerve such records have been published by Schriever and Cebulla (1938) who also found in frog's nerve the bend of the curve noted by us, although it was not so marked.

2. *Accommodation in motor units*

The next step in the work clearly required some kind of restriction of the activity to smaller populations and the simplest method (Skoglund, 1942) seemed to be to insert fine electrodes in the muscle. Silver pins in a glass tube, drawn out in a flame with the glass to a thin point, were used as different electrodes (*musc. tib. ant.*, cat) the indifferent electrode being on the bone clamped to the myograph drill. The stimulating electrodes were on the peripheral stump of the severed sciatic.

To judge from the literature on "muscle spikes" many workers are under the impression that small electrodes restrict the discharge to single motor units. But it can easily be shown, if a motor nerve be stimulated with a neon-stimulator at different strengths, that the seemingly isolated spikes of activity of constant size increase in size within a range of

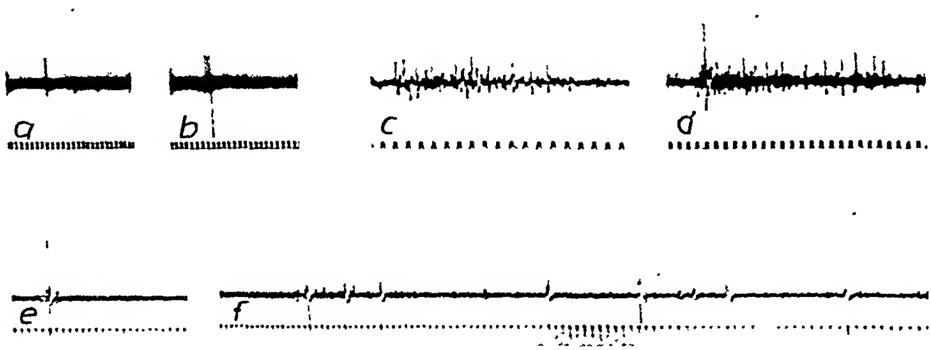


FIG. 5. Spikes recorded with microelectrode in cat's *tib. ant.* and stimulating electrodes on severed sciatic nerve. Time in 1/50 sec. Full description in text. (We regret that good films have not been obtainable.)

10 times or more when the stimuli are strengthened. Obviously therefore synchronized activity can throw into action a greater or smaller number of muscle fibres and we have no means of deciding by merely relying upon the electrode whether these fibres belong to the same motor unit or not. The all-or-none law is no reliable criterion since well synchronized spikes keep constant for a given current strength. The same holds good for recording with concentric needle electrodes (cf. Blake, Pritchard, 1930). Despite this microelectrodes are a definite improvement and some restriction of activity is obtained with them.

With this method one of us (C. R. S.) found that the motor nerve typically activates small or large spikes in the muscle (see Fig. 5) and that these have different properties with regard to accommodation. His analysis of the properties of the "spike patterns" will be published separately (Skoglund, 1942), and we shall here merely discuss *breakdown* of accommodation in relation to this new index of activity.

Figure 5a shows a single small spike at relative threshold-strength 8 and time 10, in fact the first point plotted on the lower curve of Fig. 6. Keeping the same spike as constant index this lower curve was traced in the usual manner. If at 10 msec. the strength of current was increased, the response b of Fig. 5 was obtained. This shows a large spike. The small spike now

reacts repetitively. But it is of course also possible to trace an accommodation curve with the single large spike as index. This is the upper curve of Fig. 6. The kind of response obtained for 20 msec. with the large spike at the threshold is shown in Fig. 5d. Somewhat below the threshold for the large spike, that is at strength 9.5 for 20 msec., only small spikes are seen, and there is definite repetitive firing (5c).

It would be a mistake to believe that only the small spikes are repeated. The lower curve of Fig. 5 is from another experiment, in which for 200 msec. a large spike appears at the threshold (e). The small spikes are here very small. Record f shows a small increase of current strength, from 35 in record e to 40 in f. The large spike now has become repetitive. Thus, it is only necessary to increase current strength for a given rate of rise a little above the value given by the ordinate on the accommondation curve to find a repetitive discharge in the nerve.

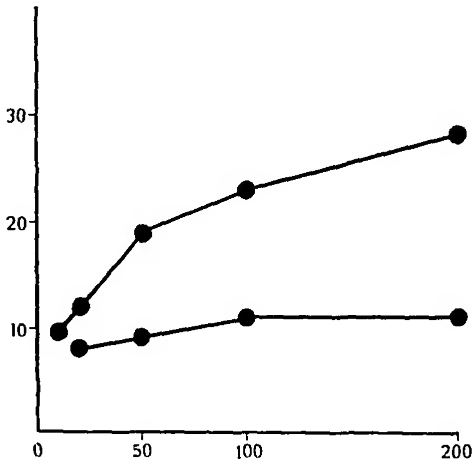


FIG. 6. "Accommodation curves" with (lower curve) small spike (a in Fig. 5) and (upper curve) large spike (b in Fig. 5) as index. Abscissae as in Fig. 1–4. Ordinates: relative current strength (*not* multiples of rheobase) in order to show relative threshold.

Accepting the result of Skoglund's (1942) analysis, *viz.* that the difference between large and small spikes depends upon the degree of "grouped action" of the stimulated fibres, we have now to reconsider repetitive firing and the breakdown of accommodation from this point of view. It is clear that *in general* repetitive firing takes place in the region above the accommodation curve, provided that relatively slowly rising stimuli be used. This proviso is necessary because with strong and rapidly rising stimuli there is an *alternative* to repetitive firing in the possibility of grouped action, or, rather, increased degree of grouped action, the small spikes probably also being due to smaller groups. The consequence of this alternative is that it is possible to plot a new and steeper accommodation curve for the grouped fibres with consequent smaller value for λ. Breakdown of accommodation as well as accommodation itself is therefore influenced by the manner in which the fibres co-operate.

The new "constant" λ which, as we now may suggest, is constant only for relatively brief rising times means that the grouped fibres resist breakdown better than relatively isolated elements,—a conclusion with many significant consequences, among them that the theoretically correct value for λ with mammalian nerves can be obtained only with isolated fibres under the electrodes.—From the point of view of sense-organs we must conclude

that *an end-organ, developing generator potential, has a much better chance of causing breakdown than any electrical stimulus over a group of elements. It has a chance of exciting a single axon in a highly adequate manner by electrotonic spread designed to minimize accommodative resistance.*

Returning now to the curves of Fig. 1–4, obtained in the usual manner with a threshold muscle contraction as index, it is clear that the variability which is so obvious a feature of such measurements to a large extent must be due to the uncertainty of the index which in turn also depends upon the degree of "grouped action," a factor so far definable only with respect to the strength of the stimulus but probably influenced by a number of other variables, among them temperature, maltreatment of the nerve etc. Thus, it is reasonable to suggest that, when in Fig. 3 a shift of the stimulating electrodes to the cut end of the nerve leads to steeper initial rise of the curves followed by sudden breakdown of accommodation, the cause behind this change is, that grouped action with consequent steeper rise is favoured by the injury potential but that at the same time the strength of catelectrotonus necessary for complete breakdown has decreased.

This raises the question as to what extent the breakdown obtained with mammalian nerves is a normal phenomenon. The small and large spikes, forming a whole "spike spectrum" and the main phenomena are certainly obtained also with unsevered nerve, just isolated and put on the electrodes. But there is no doubt but that breakdown of accommodation and small slopes of the curves are favoured by a departure of the nerve from "normality."

3. *Definition of breakdown of accommodation*

In the discussion following upon the publication of Hill's theory (1936) it has been emphasized by M. and L. Lapicque (1937, a, b, 1938) that the autorhythmic activity of nerve may have complicated measurements of accommodation, particularly in citrated-nerve, and that stimuli of long duration alter the state of the tissue. The latter objection need not be true. If care be taken to preserve an accurate index of accommodation the curves obtained with mammalian nerves are constant for hours.

But the rhythmic activity does introduce complications (cf. v. Kries, 1884, Hoffmann, 1910, Schriever and Cebulla, 1938) inasmuch as it complicates evaluation of the index, and probably, as held by Fabre (1931, 1936), represents a physicochemical system with properties very different from those tested by the usual shock technique. It is no exaggeration to state that this latter technique, as applied to whole nerve and muscle, has served to keep the facts connected with repetitive firing out of focus.

From the point of view of the problem "generator potential-repetitively firing axon," so clearly raised by all the work on the retina (cf. particularly Bernhard, 1941), the breakdown of accommodation in nerve is of interest as a model. Its definition by the aid of our results is based on the change of slope of a curve which *as a whole* serves to characterize these properties of the excitable tissue. The inverse value of the initial part, Hill's constant λ,

may still serve as a useful approach to many problems as it has done to our own work. Looking very generally upon accommodation as a process tending to counteract the effect of a maintained stimulus, our curves mean that this process, so to speak, breaks down during the time the stimulus is applied, provided that it has reached a certain level of strength, and that finally there is so little accommodation left that the stimulus always excites, no matter how slowly it reaches this level. The slope of the curve then becomes horizontal. By following our curves far enough we reach the point when catelectrotonus excites independently of and not counteracted by accommodation. Above our curves lies the region of repetitive firing of the particular single unit or grouped unit concerned. We do not believe ourselves that spikes of the small type represent single fibres.

4. *Inhibition*

In looking for a model reproducing retinal inhibition it has been necessary to discard the idea that it could be explained as a subnormal phase according to Gasser (in Erlanger and Gasser, 1937). In 1935 Granit and Therman showed that inhibition of the discharge in the optic nerve is preceded by a slow potential belonging to the component PIII of the retinal response. This result was subsequently confirmed by Hartline (1938). A full discussion of retinal inhibition is found in a summary by Granit (1938). Since then Therman (1938) has shown that the retinal component does not agree with subnormality by any of the chemical tests selectively influencing the positive after-potential. Bernhard and Skoglund (1941) have found that alcohol selectively diminishes the negative component PIII and that at the same time inhibition also diminishes. The connexion between PIII and inhibition has thus been confirmed. This does not necessarily mean that PIII could be excluded as factor causing excitation. This potential precedes PII at both "on" and "off" as we have now definitely proved (Bernhard, unpublished work).

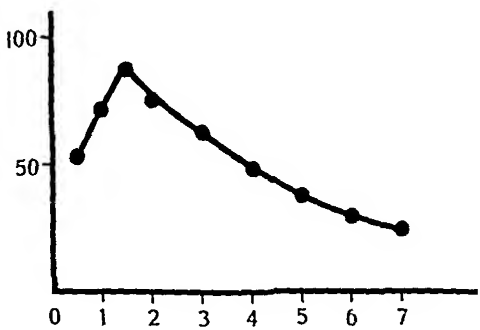


FIG. 7. Frequency of discharge in frog's nerve following removal of anelectrotonic block. Abscissae: time in sec. Ordinates: frequency per sec.

If PIII were an anelectrotonic state developing during illumination, then the off-effect could be regarded as the consequence of the release of anelectrotonus which during illumination had piled up inhibition influencing certain retinal neurons. Our model would then be an anelectrotonic block. Actually, after an anelectrotonic block across a frog's nerve, the impulse frequency of the ensuing discharge often follows a curve of the type given by the retinal off-effect. This is shown in the example of Fig. 7.

On the other hand, it is known that a sufficiently strong catelectrotonus also may block nerve fibres (see *e.g.* Bugnard and Hill, 1935). In our experiments catelectrotonic inhibition has often been seen when for some reason or other the nerve fires spontaneously as in the record of Fig. 8. Here the discharge is inhibited by a catelectrotonus. The component PIII could also just as well be imitated by this model. It is known that the retina is oppositely influenced by opposite polarizing potentials across it (Granit and Helme, 1939). At the moment it is hardly necessary to go beyond the general hypothesis that a generator potential, capable of causing excitation, may also be capable of causing inhibition. Below we shall show that the same polarizing current gives excitation *or* inhibition in the optic ganglion of *Dytiscus* depending upon whether it is silent or discharges spontaneously at the time of applying the current.

5. *Latent period and adaptation*

Both phenomena are reproduced by the model. The slower the rise of the current the later the initiation of the iterative discharge. This follows directly

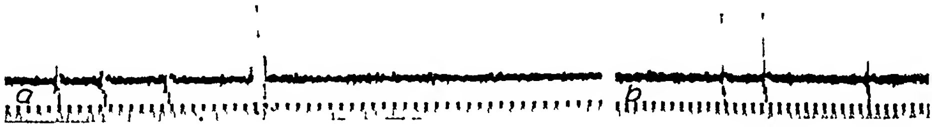


FIG. 8. Spontaneous discharge (a) in cat's *tib. ant.* muscle. Catelectrotonus on sciatic nerve leading to an outburst of muscle spikes followed by inhibition for the time the stimulus remains on. Afterwards (b) renewal of spontaneous discharge.

from the fact that a certain minimal strength of current is necessary for it, and that it is possible, by delaying the rate of rise of the current, to postpone the moment at which this level of excitation is reached. When passing beyond the strength of current necessary for iterative firing anywhere above the accommodation curve, it is seen that iterative firing does not continue indefinitely but stops after some time (Skoglund, 1942). There is thus a parallel to adaptation, which, of course, in addition may be determined by the properties of the primary mechanism which precedes the generator potential of the end-organ.

6. *Application of model to eye of Dytiscus*

The primary visual cells of both vertebrate and invertebrate eyes are homologous and have been carried through the whole progress of evolution (Kappers, Huber, and Crosby, 1936). In the water-beetle Bernhard's recent work (1941) has shown that the visual cells easily can be separated from the optic ganglion lying behind them and that the isolated retina gives a large smooth action potential reminiscent of the simple response of *Limulus* (Hartline, 1928, Hartline and Graham, 1932), and preceding the discharge in the nerve. If the ganglion is included spikes or synchronized waves appear on the response (cf. Adrian, 1932, 1937).

Where in this sense-organ are impulses generated? In order to answer this question we attempted to record from various parts of the retina by means of microelectrodes, *e.g.* through openings in the chitinous cover of the lenses and from behind. But from nowhere in the retina can any trace of impulses be obtained, not even if the microelectrode is forced in between optic ganglion and retina with tip towards the latter. In contradistinction to this stands the fact that impulses are obtained practically everywhere from the ganglion or on the optic nerve emanating from it.

From the work of Granit and Therman (1938) and that of Bernhard (1940, 1941) we know that the large potential difference developing across

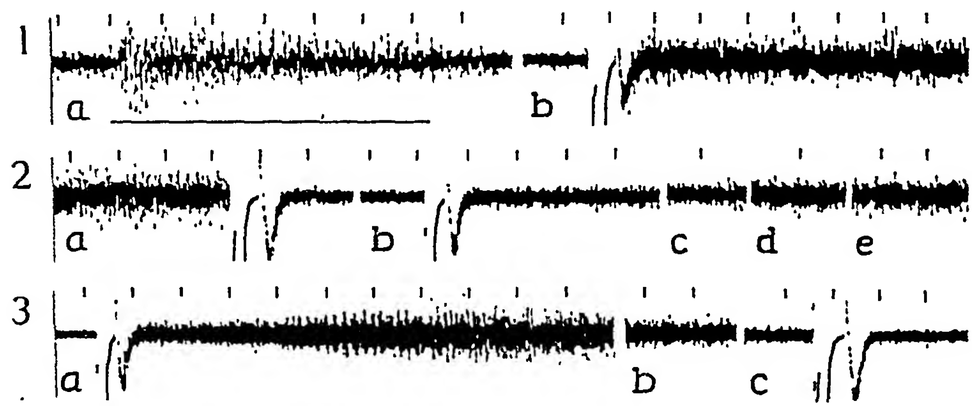


FIG. 9. Eye of *Dytiscus*. 1a, whole eye illuminated, optic nerve. 1b, isolated silent ganglion stimulated cathodally (at artefact). 2a, activity in isolated spontaneously active optic ganglion inhibited by cathodal stimulation, 2b, removal of catelectrotonus, 2c, d, e, f, gradually recovering spontaneous discharge. 3a, silent isolated optic ganglion stimulated anodally, 3b, gradual diminution of frequency, 3c, removal of anelectronus. All records taken with small metal electrodes. Time in $\frac{1}{5}$ sec.

the retina upon illumination is carried down electrotonically to and over the ganglion. On our hypothesis this would serve as generator mechanism for impulses, provided that the threshold for breakdown of accommodation were low. That this must be the case is shown by the tendency of the eye to discharge spontaneously. Fig. 9 shows an experiment with a cathode or anode on the ganglion isolated in air and the other electrode on the supraoesophageal ganglion. Microelectrodes are used for picking up the impulses from the ganglion.

First follows a control with illumination (1a) showing that the retina was alive before isolated from its ganglion. There is a small spontaneous discharge before illumination sets in. After separation from the retina the optic ganglion is stimulated cathodally with anode on the supraoesophageal ganglion. This leads to a discharge (1b). Record 2a shows another spontaneously active ganglion. Cathodal stimulation in this case immediately blocks the discharge which slowly reappears when the current is cut off (2b, c, d, e). Finally anodal stimulation (3a) is applied onto another isolated optic gan-

glion which was silent from the start but began to discharge upon stimulation. This discharge gradually diminished and did not reappear when the stimulus was removed (3c).

Now a preparation of this type consisting of ganglion cells surrounding a bundle of nerves emanating at one end as an optic nerve cannot be precisely stimulated anodally or cathodally. But the experiments nervertheless show that in both cases both inhibitory and excitatory effects were obtained. The only rule observed was the one illustrated, *viz.* that silent ganglions began to discharge and active ones were inhibited by the polarizing current.

Actually in this eye both effects are also observed by stimulation with light. When the "generator" potential develops across the visual cells upon illumination, there follows a heavy outburst of impulses which instantaneously stops at "off." But if the preparation is discharging spontaneously it can be seen that the drop of potential at "off" leads to an additional slow potential from the ganglion accompanied by a temporary inhibition of the spontaneous discharge (Bernhard, 1941). It is therefore possible that the abrupt stop of the normal discharge following cessation of illumination also is brought about by active inhibition.

These experiments together with those of Bernhard (1941b) show: (i) the sensory cells themselves develop only generator potential but no impulses, (ii) the generator potential is carried down electrotonically to the optic ganglion, where both a slow stationary potential difference as well as an impulse discharge is initiated, (iii) in some cases inhibition turns up instead of excitation, and (iv) these phenomena all can be imitated by polarizing currents (cf. also Granit and Helme, 1939). Whether the large potential difference across the sensory cells initiates impulses directly or *via* the slow potential in the ganglion cells (Bernhard, 1941), cannot be discussed without further experimentation.

Other sense-organs may be working on the same principles, unless so primitive that breakdown of accommodation takes place directly in free thin nerve-endings influenced by changes in ion balance or the concentration of surface-active substances released around them. (*e.g.* pain).

Summary

The work described serves the twofold aim of clarifying some elementary questions regarding accommodation in nerve relative to repetitive firing and of finding out to what extent a nerve discharging repetitively in response to slowly rising electrical stimuli can serve as model sense-organ.

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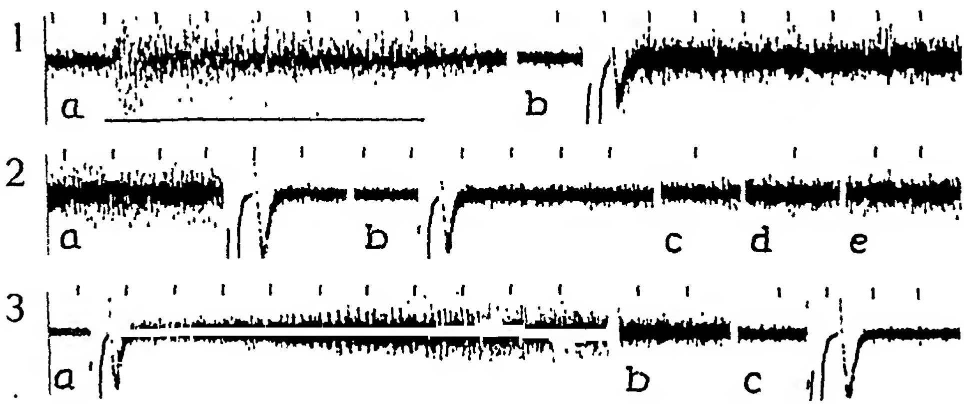

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In order to obtain a better defined index, microelectrodes were inserted in the muscle and isolated spikes, elicited by stimulation of the motor nerve with slowly rising currents, are used as the constant index necessary for measuring accommodation correctly.

Breakdown of accommodation is shown to take place also with this index and is redefined in terms of the properties of single spikes. It is shown that "grouped activity" in which several fibres fire synchronously is an alternative to breakdown or accommodation and that the accommodation curves are determined by the degree of interaction of the stimulated fibres.

In the region above the accommodation curves the nerves fire repetitively.

Not only anodal but also cathodal polarization caused by the slowly rising stimuli is capable of inhibiting a spontaneous discharge in the nerve.

The significance of these results is discussed from the point of view of nerve as model sense-organ on the assumption that a slow "generator potential" is the mechanism which in sensory end-organs fires the axon repetitively.

Application of the model to the retina and optic ganglion of *Dytiscus* shows that both excitation and inhibition may be obtained by anodal and cathodal polarization of the ganglion and that no impulses but only a slow potential is generated within the isolated retina of this animal. A silent isolated optic ganglion is excited, and a spontaneously firing one, inhibited by the polarizing current.

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THE EFFECT OF SECTION OF THE MEDIAL LEMNISCUS ON PROPRIOCEPTIVE FUNCTIONS IN CHIMPANZEES AND MONKEYS

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INTRODUCTION

THE ANATOMICAL observations of Le Gros Clark (1, 2) and Walker (8) have demonstrated the course and termination in the primate thalamus of the fibers of the medial lemniscus and dentato-rubro-thalamic tract. These tracts constitute separate afferent pathways which end discretely in the latero-ventral nucleus of the thalamus whence they are relayed to the cortical post and pre-central areas respectively. While both systems are known to carry proprioceptive impulses, the functional relationship of the fillet and the cerebellar projection to the thalamus has not been established. In the present paper an attempt was made to study the alterations in the proprioceptive skill and weight discriminatory ability of chimpanzees and monkeys following interruption of these tracts and to compare the resulting deficit with that observed after lesions at other sensory levels of the central nervous system.

METHOD

In two chimpanzees and eight *Macaca mulatta* the left medial lemniscus was sectioned at the level of the inferior colliculus, just rostral to the decussation of the brachium conjunctivum. In ,two mangabeys, *Cercocebus torquatus atys*, the superior cerebellar peduncle bearing the dentato-thalamic fibers was severed. In one chimpanzee and several of the macaques with interruption of the mesial fillet, the ascending dentato-rubrothalmic tract was also involved. All operations were carried out under sodium amytal anaesthesia with sterile technique and the animals were observed for periods ranging from three weeks to eighteen months.

The chimpanzees, mangabeys, and one macaque, "Dynamite," were trained to discriminate weights according to the technique described by Ruch (6, 7). Pre- and postoperative limens and comparison of the normal and affected limbs after operation, afforded a quantitative index of this proprioceptive function. Clinical observations of running, climbing and cage activities as feeding and grooming, furnished valuable indications of the measure of proprioceptive skill. Placing and hopping reactions were studied in all of the monkeys, as was the "voluntary" grasp response to the placing of the examiner's finger in the animal's hand or foot. Observations were also made of changes in motor power, resistance to passive motion, reactions to thermal and painful stimuli, pupillary changes and disturbance of piloerection.

All operative lesions were verified by histological examination. The Weil and Nissl methods were employed in most cases. Four monkeys were sacrificed at the end of 21 days for study of Marchi degeneration.

OBSERVATIONS

The principal observations are covered in the following protocols. *Experiment 14. Section of left medial lemniscus and lateral spinothalamic tracts. Sparing of dentato-thalamic fibers.*

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Persistent hemianalgesia. Marked initial defect in proprioceptive function which rapidly improved. Ability to discriminate weights normal two months after operation (Alice).

The subject of this experiment was a healthy, docile young female chimpanzee.

First operation: On August 3, 1939, under sodium amytal anaesthesia, the left mesial lemniscus was severed at the level of the inferior colliculus. The temporal lobe was elevated, the cisterna ambiens opened, and a stab made just dorsal to the groove separating the cerebral peduncle from the tectum mesencephali.

Postoperative notes: On the first postoperative day the chimpanzee was able to move its right limbs slightly, but could not grasp. The right extremities could be placed by the examiner in any awkward position, without the animal correcting the abnormal posture. The deep reflexes were not increased and there was no Babinski. The animal did not respond to pinprick over the entire right side of the body and face, while a much more gentle stimulation on the left caused vocalization and prompt withdrawal. Examination of the cranial nerves showed the corneal reflexes to be present bilaterally, but somewhat diminished on the right. The left pupil was larger than the right and there was an apparent right sided hemianopia. There was a horizontal nystagmus with the quick phase to the left. A slight supranuclear facial weakness was seen. In the next few days the hemianopia, nystagmus and facial weakness disappeared. The sensory deficit persisted, while there was a rapid return of motor power. The right limbs were moved well, but were of little use in feeding and climbing, apparently due to a loss of touch and position sense. The chimpanzee was able to walk but did so with a characteristic high stepping of the affected extremities. There was still no response to pinprick and pinching of the right side. No change in resistance to passive motion of the limbs was noted.

The subsequent three weeks saw a progressive return of proprioceptive function. The animal grasped with either hand. It could, one month postoperatively, manipulate even a small bit of peanut in acceptable fashion. Gait still showed a slightly higher stepping with the right leg. The animal was fond of trying to slip a key into a lock and regained its skill at this. Six weeks after operation, training in weight discrimination was resumed and the animal soon performed at the preoperative level.

The animal died on January 11, 1940 after an attempt to add to the proprioceptive deficit by sectioning the right superior cerebellar peduncle. The brain stem was sectioned serially and studied by the Weigert and Nissl methods. At the level of the inferior colliculus, the left mesial fillet was sectioned except for the narrow mesial tip; the left lateral spinothalamic tract was completely interrupted. There was a slight amount of damage to the brachium conjunctivum at the rostral part of the decussation. The pyramids were spared. There was also injury to a few of the fibers of the left third nerve. In sections up through the level of the red nucleus, the left medial meniscus was seen to be completely demyelinated.

Experiment 15. Section of left medial meniscus and ascending dentato-rubro-thalamic fibers. Intention tremor of left arm. Permanent deficiency in proprioceptive skill and ability to discriminate weights. Slight right hemihypalgesia. (Johnnie.)

The subject of this experiment was a playful young male chimpanzee.

First operation: On August 7, 1939, a left-sided, mid-brain tractotomy was attempted. The following day, it was apparent that the ascending sensory tracts had not been interrupted as the animal used both hands well and responded promptly to pinprick stimulation. Accordingly, the chimpanzee was reoperated.

Second operation. On August 11, 1939, under sodium amytal anaesthesia, the flap was reopened and the stab in the midbrain exposed and enlarged to a depth of 10–11 mm. The animal withstood this second procedure excellently.

Postoperative notes. On the first postoperative day, the right limbs hung limply, with diminished resistance to passive movement, though the animal was able to use them to some extent as a support. In attempting to walk, it tended to drag the affected extremities along after it. Voluntary grasp was absent in the right hand and abnormal postures were not corrected. The rest of the sensory status was difficult to evaluate. The chimpanzee was frequently seen to scratch the right side of its body as if there were some paresthesia. Pricking the right limbs with a pin resulted in a delayed response, while several seconds after the application of the stimulus the animal would rub the affected spot. On the left side there was prompt withdrawal to any noxious stimulation.

The disability of the right limbs persisted, though it was evident that this impairment was not due to motor weakness as the animal moved all four limbs well. In resisting dis-

agreeable procedures, as a scrubbing for a skin infection, it struggled powerfully. On September 13, 1939, one month after the second operation, the following note was made: "There is marked disability of the right limbs. The animal is able walk and climb about the cage but the right limbs are of little assistance. In walking, the right upper extremity is carried in flexion at the elbow, the hand swings flail-like at the wrist, while the right lower limb is raised high in an exaggerated stepping. In climbing, the subject repeatedly misses its footing with the affected hand and foot. It gets into awkward positions with the right leg bent back behind it. It does not take food with the right hand or grip the examiner's fingers. In attempting these acts, an intention tremor of large amplitude is present. There appears to be diminished resistance to passive motion in the right limbs. The animal apparently perceives thermal and painful stimuli over the right side of the body, though the responses are diminished compared to the left. The spinothalamic sensory deficit is not as severe as in chimpanzee Alice. The withdrawal movements illustrate the good motor power of the right limbs. The knee jerks are lively and active. The left pupil is larger than the right."

The animal was disinclined to use the right limbs but when forced to do so began to show a slight improvement. It became able to grasp and could clumsily convey food to its mouth, but could not manipulate small objects as a peanut kernel, with which it would fumble. The intention tremor persisted.

Preoperative training had resulted in the chimpanzee discriminating a 40 per cent weight difference 98 per cent correctly, and a 20 per cent weight difference with a score of 92 per cent. One month after the operative procedure, Johnnie was only 60 per cent correct at a 40 per cent weight difference, and at a 30 per cent difference gave a 50 per cent score. Despite the intention tremor and the clumsiness, it was able to effectively grasp the cannisters containing the weights, so that the motor disability was not responsible for the defect.

Experiment 21. Section of left superior cerebellar peduncle. Marked intention tremor and ataxia of left hand. No loss of manipulative skill and ability to discriminate weights. Placing and hopping reactions retained.

The subject of this experiment was a male sooty mangabey (*Cercocebus torquatus atys*) weighing 3400 grams.

First operation. On October 31, 1939, under sodium amytal anaesthesia, the left superior cerebellar peduncle was severed. The cisterna ambiens was opened and the peduncle cut as it entered the brain stem.

On the first postoperative day, the animal did not use the contralateral (right extremities) which were dragged about the cage. There was a severe intention tremor and ataxia of the homolateral left upper limb. When the animal attempted to convey food to its mouth with the left hand, it would often hit itself in the right eye. When the left arm was supported, however, the monkey was able to pick up small objects and manipulate them quite well.

A right-sided visual field defect was present. This cleared up along with the right hemiparesis after two or three days. A progress note of November 7th read "The severe left-sided ataxia, dysmetria and intention tremor persist. In running, the left limbs swing wildly. Placing and hopping reactions are present bilaterally. The animal uses the right limbs to hold the left hand and control the tremor. When this is done it is seen that the monkey is able to manipulate bits of food in acceptable fashion. Training in weight discrimination was resumed one month after operation. Despite the ataxia and intention tremor, there was no change from the preoperative level."

Section of Medial Lemniscus in Monkeys

In the macaques, the following observations were made after section of the left medial lemniscus at the midbrain level.

On the day after operation, the monkey sat in its cage with its head inclined to the right, the chin slightly rotated to the left. There was a transitory rotary nystagmus, which with the abnormal head posture cleared up in two or three days. The limbs contralateral to the lesion hung limply with obviously complete loss of propioceptive function. The affected arm and

leg fell into awkward postures or became entangled in the wire mesh of the cage without any attempt at correction.

On the fifth postoperative day, the animal was released from the cage and allowed to run down a long corridor. Here a right sided visual field defect was apparent as the monkey repeatedly collided with the right wall. There was a characteristic, high stepping, galloping gait in which the right limbs were flung in a high arc. Climbing was performed poorly, the animal missing its perch with the right limbs. This clumsiness was best observed when the left normal limbs were restrained and the animal forced to feed itself with the right hand. The monkey would have great difficulty in grasping even a large morsel, fumbling, and being quite unable to manipulate it. This disability could not be attributed to weakness, as there was good power in the affected limbs as observed when the animal struggled to free itself from restraint.

The degree of recovery in the affected limbs after section of the mesial fillet appeared to depend, as in the chimpanzees, on the extent of the additional involvement of ascending dentato-thalamic fibers. In the macaques in whom there had been no damage or only slight implication of the brachium conjunctivum, there was a rapid return of function, so that three weeks after operation, only a slight proprioceptive defect remained. There was some disinclination to use the affected extremities with a tendency to overstepping still noticeable in the gait. The animals climbed well, though the surefooted scampering and abandon of the normal macaque had given way to a degree of caution. The animal carried food to its mouth easily without ataxia of the involved hand, though when its attention was turned elsewhere, the monkey would occasionally drop the morsel it was holding. Grooming activities were performed deftly and while there was difficulty in manipulating very small bits of peanut, whole kernels were scooped up well.

After complete section of the fillet, placing and hopping reactions were permanently absent in the contralateral limbs whether or not the dentato-thalamic fibers were also involved. "Voluntary" grasp, as distinguished from forced grasping, such as follows lesions of the premotor cortex, was also absent in the affected limbs. When the examiner's finger was placed in the animal's hand it responded by grasping only on the normal side. The retention or loss of the placing and hopping and grasp reflexes proved to be a valuable clinical index of the completeness of the procedure interrupting the mesial fillet.

The stab severing the lemniscus also destroyed lateral reticular substance in the midbrain. This interruption of descending autonomic pathways was manifested clinically by the development of a homolateral piloparalysis. This appeared from one to four months after the operation. When the animal was chased or otherwise frightened, the hair on the opposite side of the body ruffed up while that on the homolateral side remained flat with a sharp midline demarcation. Horner's syndrome was never observed in either chim-

panzees or monkeys. The pupil on the operated side was generally larger, due presumably to injury to third nerve fibers.

Discussion

The findings show that in chimpanzees and monkeys a considerable degree of skill in weight discrimination and general propioceptive ability may be regained after section of the contralateral medial lemniscus. This is surprising because the fillet is regarded as the great afferent pathway carrying proprioceptive impulses from the gracile and cuneate nucleii to the thalamus. That such recovery of function may be mediated through the dentato-rubro-thalamic tract is suggested by the fact that when the operative lesion involved both the fillet and the adjacent crossed fibers of the brachium conjunctivum little improvement ensued. Despite the large projections received from the posterior columns, it is generally accepted that the cerebellum subserves only unconscious proprioceptive adjustments. Even with large cerebellar defects in man, conscious proprioceptive sensation remains intact and Holmes (4) found no disturbance in the faculty of appreciating and discriminating weights in patients with cerebellar injuries. Similarly in our own experiments, primary section of the superior cerebellar peduncle produced no diminution in the ability of monkeys to discriminate weights even though the performance was handicapped by ataxia and intention tremor. It would appear that cerebellar fibers are concerned in the maintenance of the function of weight discrimination and may compensate after interruption of the pathway normally mediating conscious proprioceptive impulses.

Clark (1) and Walker (8) have pointed out the close anatomic association of the medial lemniscus and dentato-rubro-thalamic tracts. They constitute separate fiber systems which terminate in close juxtaposition but discretely in the latero-ventral thalamic nucleus. Ferraro and Barrera (3) have recognized the physiological significance of this dual proprioceptive system at a lower afferent level. These authors found that in monkeys, greater disability was produced by sectioning the fasciculus cuneatus than by destroying the nucleus cuneatus. This was explained by the fact that the interruption of the column cut off fibers destined for both the cerebellum and mesial fillet while the nuclear lesion involved only fillet connections and spared the cerebellar fibers.

It is also of interest to compare the findings with those observed after cortical lesions. It has been demonstrated (1, 2, 8) that there is a double projection of thalamic-cortical fibers. The area of the latero-ventral thalamic nucleus that receives the fibers of the medial lemniscus is the source of the relay to the postcentral gyrus, while the region of the ending of the cerebellar fibers sends impulses to the precentral area. With lesions of the frontal lobe in man (8), retrograde degeneration occurred in the contralateral dentate nucleus. Studies from this laboratory (6, 7) have shown that the postcentral gyrus may be removed in primates without lasting impairment in

the ability to discriminate weights or degrees of roughness. Even after ablation of the parietal lobe in monkeys and chimpanzees, a considerable degree of function could be regained with sufficient lapse of time and retraining. The failure of postcentral ablation to produce a significant deficit, corresponds to the result of medial lemniscus section, and bears out Head's dictum that the thalamus does not play a major role in the recovery of proprioceptive function after parietal lesions. Parietal lobectomy is somewhat more effective in producing a deficit than mesial fillet interruption. This is explicable on the grounds that the posterior parietal lobule is in communication with both ascending sensory systems (Ruch, personal communication). It is likely then, that fibers of cerebellar origin may subserve the recovery of proprioceptive function following parietal lesions as they do at lower levels of the central nervous system.

Conclusions

1. Section of the medial lemniscus in monkeys and chimpanzees did not produce an enduring loss of proprioceptive skill in the ability of trained animals to discriminate weights.
2. Primary section of the superior cerebellar peduncle carrying dentato-thalamic fibers produced no significant alteration in weight discrimination.
3. When lesions of the medial lemniscus also involved the crossed dentato-rubro-thalamic tract, there was a marked permanent loss in general proprioceptive function and the ability to discriminate weights.
4. It is suggested that the cerebellar projections to the thalamus may subserve the recovery of proprioceptive function following interruption of the medial lemniscus.

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EXCITATION AND INHIBITION OF PHRENIC MOTOR NEURONES*

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STUDIES of the activities of individual respiratory motor neurones by Adrian and Bronk (1928), Bronk and Ferguson (1935) and Gesell, Atkinson and Brown (1941) have shown that inspiratory activity in both phrenic and intercostal motor units is graded in three ways: by variation in the frequency of discharge of the several motor units; by variation in the numbers of motor units active; and by duration of activity of motor units. From these studies certain inferences have been made as to the nature of the discharge of neurones making up the respiratory center, though of course these neurones lie one or more levels upstream from the ones whose behavior was actually characterized. The localization of the respiratory center, and its differentiation into inspiratory and expiratory divisions (Pitts, Magoun and Ranson, 1939a; Beaton and Magoun, 1941; Pitts, 1941) make possible a study of the behavior of respiratory motor neurones under controlled excitation of the centers. The hope that such a study would add further to an understanding of the functions of the respiratory center and the respiratory motor neurones as well, prompted the present investigation.

METHODS

Our experiments have been performed on cats anaesthetized with 30 mg. per kg. of nembutal intravenously, supplemented as needed with additional small doses. All studies have been made on units dissected from the third cervical root of the phrenic nerve. If a sufficient length of nerve could be exposed, the fourth root was spared, otherwise it was sacrificed. The nerve was carefully and repeatedly split until only one or two fibers remained active within the strand under observation. Potentials were amplified by a condenser coupled amplifier and recorded on bromide paper with a G. E. mirror oscillograph. The animal was maintained in a warmed and humidified shielding box. The respiratory center was stimulated through bipolar needle electrodes oriented in the Horsley-Clarke stereotaxic instrument, the stimuli being brief repetitive condenser discharges (time constant approximately 0.1 msec.), independently variable as to frequency and intensity. Respiration was recorded by a light rubber optical tambour recording pressure changes within a five gallon bottle connected through a soda lime tube to the tracheal cannula. The system was filled with oxygen and except during the taking of records was constantly replenished by opening to a rubber bag oxygen reservoir.

RESULTS

Nature of phrenic neurone activity

During the inspiratory phase of eupneic respiration, phrenic neurones respond repetitively with a slowly augmenting frequency of discharge, which suffers sudden decrement as expiration begins. A certain number of neurones are active throughout the inspiratory cycle, as is the neurone of

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the control record of Fig. 3. Others come into activity for a brief interval only near the peak of inspiration (control record, Fig. 1). As Gesell, Atkinson and Brown (1941) have pointed out, increase in frequency of discharge and successive recruitment during development of inspiration, release additional inspiratory energy smoothly as it is needed (control record, Fig. 5). Only a fraction of the phrenic neurones, however, is active during eupneic respiration and the discharge frequencies which they attain are relatively low.

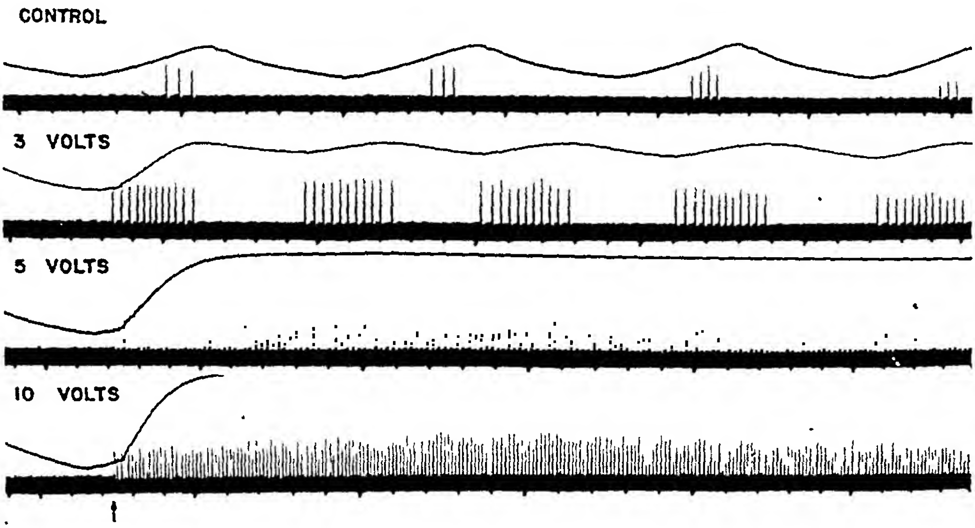

FIG. 1. Impulses discharged by a single phrenic neurone and respiratory response on stimulation of the inspiratory center with stimuli at a frequency of 220 per sec. and intensities of 3, 5, and 10 V. Arrow marks beginning of stimulation. Time, $\frac{1}{5}$ sec.

Thus there exists a large reserve of inspiratory energy which may be drawn against under conditions of respiratory stress. Such conditions of stress may be simulated by electrical shocks at controlled intensities and frequencies applied to the respiratory center.

Effect of inspiratory center stimulation on phrenic neurone discharge

Stimulation of the inspiratory center with repetitive shocks of high frequency and moderate intensity (240 per sec.; 8 volts) leads to maintained deep inspiration involving both thorax and diaphragm to an essentially maximal degree (Pitts, Magoun and Ranson, 1939a). The neural basis for this respiratory response is illustrated in part by the behavior of the phrenic neurone in the lower records of Fig. 1. Stimuli of an intensity of 5 or 10 V. and a frequency of 220 per sec., applied to the inspiratory center at the time indicated by the arrow at the bottom of the records, led to repetitive discharge of the phrenic neurone and deep inspiration, both maintained for the duration of stimulation.

Factors controlling magnitude of inspiratory response

A. Stimulus intensity. It is apparent from inspection of the records of Fig. 1 that both the response frequency of the phrenic neurone and depth of inspiration are related to stimulus intensity. At intensities of 4 v. or more, phrenic neurone discharge was constant and the respiratory trace showed no phasic movements. At intensities of 2 and 3 v., however, the response of the neurone was interrupted and the respiratory trace, though shifted towards a

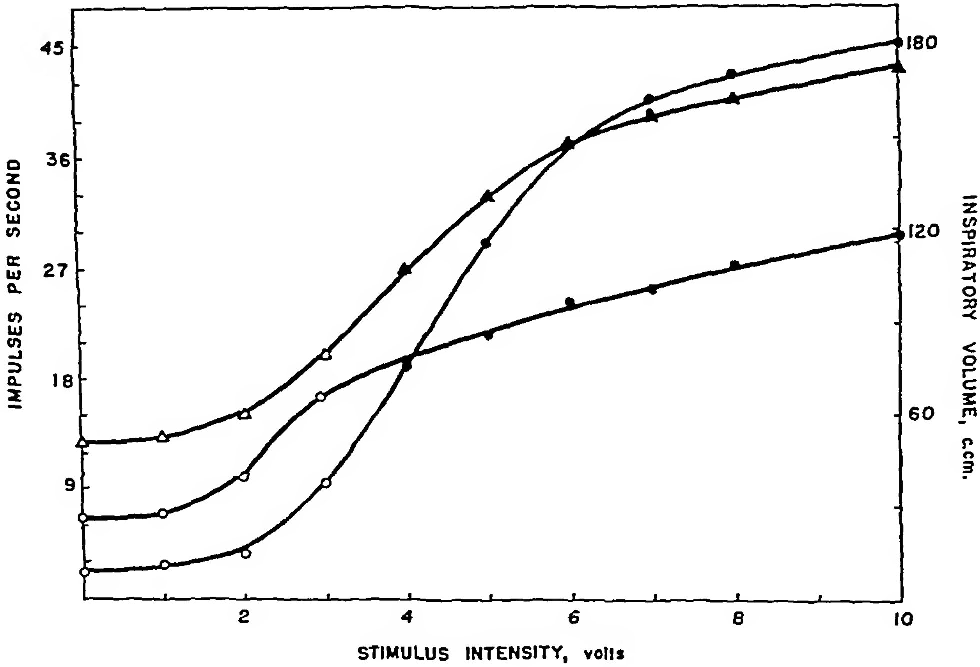

FIG. 2. Relation between the discharge of impulses by phrenic neurones, volume of inspiration and intensity of stimulation of the inspiratory center. Triangles, inspiratory volume in c. cm. measured from the level of the preceding normal expiration. Circles, impulses discharged per sec. by two phrenic neurones. Frequency of inspiratory center stimulation, 220 per sec. throughout; intensities as indicated. Hollow symbols indicate a response showing repiratory modulation; solid symbols indicate a maintained uninterrupted response.

position of inspiration, showed definite respiratory waves. An intensity of 1 v. approximated threshold, for both respiratory response and phrenic neurone discharge showed minimal augmentation.

Quantitation of the entire series of records of which Fig. 1 is but a part, yields Fig. 2. The response of first one and then a second phrenic neurone was determined to intensities of stimulation from 1 to 10 v. Stimulus frequency was kept constant throughout at 220 per sec. and the position of the electrodes was unchanged. The responses of these two phrenic neurones in terms of impulses per sec. are plotted in the lower two curves as

circles. The respiratory responses, identical of course in the two series of tests, are plotted in the upper curve as triangles. They represent depth of inspiration, in cubic centimeters, from the preceding normal expiratory level. The use of hollow symbols indicates that respiration continued rhythmic, while the solid symbols at intensities of 4 v. or more represent maintained inspiration.

The characteristic relationship between stimulus intensity and either frequency of phrenic nerve impulses or depth of inspiration is sigmoid in form. However, the two neurones of Fig. 2 do not behave in an identical manner to an increase in stimulus intensity, nor does the response of either

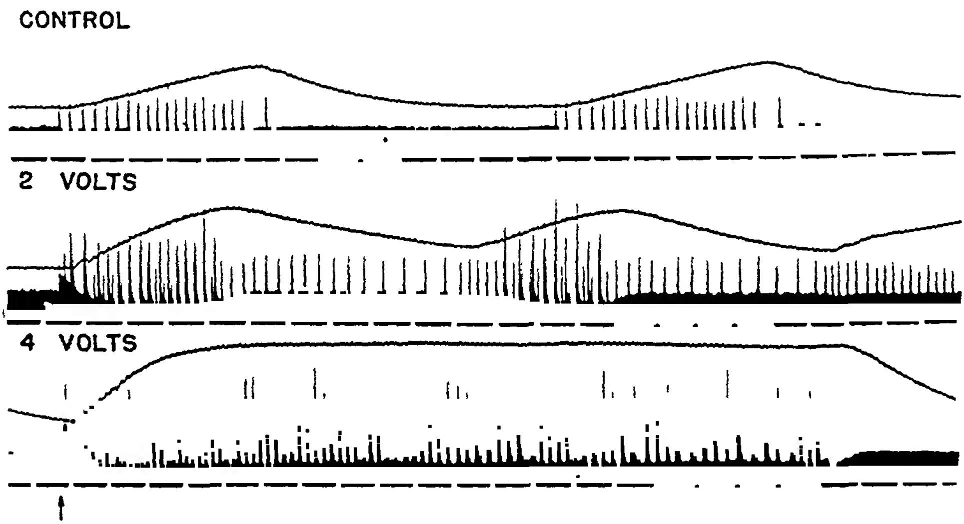

FIG. 3. Impulses discharged by two phrenic neurones and respiratory response on stimulation of the inspiratory center with stimuli at a frequency of 220 per sec. and intensities of 2 and 4 v. Arrow marks beginning of stimulation. Time, ⅕ sec.

reflect at all accurately the inspiratory volume curve. The essential features of these curves have been duplicated in five other experiments, though magnitudes and slopes vary. In general, an intensity of one volt is near threshold, the greatest change in response occurs between 3 and 6 v., and at higher intensities both inspiratory volume and frequency of impulses tend to level off. Obviously, inspiratory volume is related to frequency of discharge of the several motor neurones. However, individual neurones vary so widely in their behavior to stimuli of a given intensity, that agreement between volume and frequency curves might reasonably be expected only if one averaged the response of many neurones.

Even so, agreement would not be perfect for, as is evident from Fig. 3, numbers of neurones active, as well as the degree of activity of each, determine the inspiratory response. The preparation from which Fig. 3 was obtained consisted of two functional neurones, of which only one was spon-

taneously active. Stimulation of the inspiratory center at high frequency (220 per sec.) and low intensity (2 v.) recruited the previously inactive neurone characterized by greater spike potential. In addition, the discharge frequency of the previously active neurone was increased somewhat and its activity prolonged throughout the respiratory cycle though at a reduced frequency during expiration. As a consequence of the operation of such factors, inspiration increased in depth and a shift of respiratory mid-position toward the inspiratory side occurred. Increasing the intensity of stimulation to 4 v. increased frequency of discharge of both neurones, maintained their activities constant, and further increased inspiratory depth. Quantitation of such results is difficult for if more than two neurones are active, it becomes almost impossible to distinguish with certainty their individual impulses. However, it has been observed that there is a wide range of stimulus intensity over which additional neurones are recruited. Thus in Fig. 2, even at the highest intensities, it is probable that a portion of the inspiratory increment resulted from recruitment of additional neurones as well as from an increased frequency of discharge of those already active.

B. Stimulus frequency. If the intensity of stimulation is maintained constant and the frequency varied, results qualitatively similar to those shown in Fig. 1 and 2 are obtained. A plot of such an experiment is shown in Fig. 4, in which the behavior of first one and then a second phrenic neurone was determined to stimuli of an intensity of 8 v. and frequencies from 6 to 220 per sec. The significance of the various symbols is the same as in Fig. 2.

Both the frequency of phrenic nerve impulses and depth of inspiration increase with increasing stimulus frequency. The relationship like that for stimulus intensity is a sigmoid one. Qualitatively similar results have been obtained in four additional experiments, though magnitudes are dissimilar. By chance, no doubt, the behavior of one of the neurones of Fig. 4 approximates quite closely the inspiratory volume curve, though the other plateaus quite early in the frequency series. Again, correspondence between inspiratory volume and impulse frequency might be expected only on the basis of the average response of many neurones. As with stimulus intensity, an increase in stimulus frequency leads to the recruitment of additional neurones, and it is probable that the inspiratory increments of Fig. 4, even at the highest stimulus frequencies are in part the result of such recruitment.

The results just presented may be summarized as follows. Stimulation locally within the inspiratory center of more units, by increasing stimulus intensity, or of a given number of units at higher frequency, leads to an increased rate of discharge and recruitment of phrenic motor neurones. Such a view implies multiplicity of excitatory pathways between inspiratory center and any given final motor neurone. The degree of excitation of the neurone, as measured by the frequency at which it fires, is dependent then on the average number of impulses impinging on it from these many sources. Those neurones which are normally quiescent receive subliminal excitation (*vide infra*), but may be brought into activity by increasing either numbers of

pathways active or the frequency at which they transmit impulses. Low intensity or low frequency stimuli sum with spontaneous impulses from the inspiratory center and cause less excitable motor neurones to become active earlier in the inspiratory cycle. The inhibitory mechanisms which normally serve to interrupt spontaneous inspiratory activity (Pitts, Magoun and Ranson, 1939c) likewise inhibit this augmented discharge, so rhythmic res-

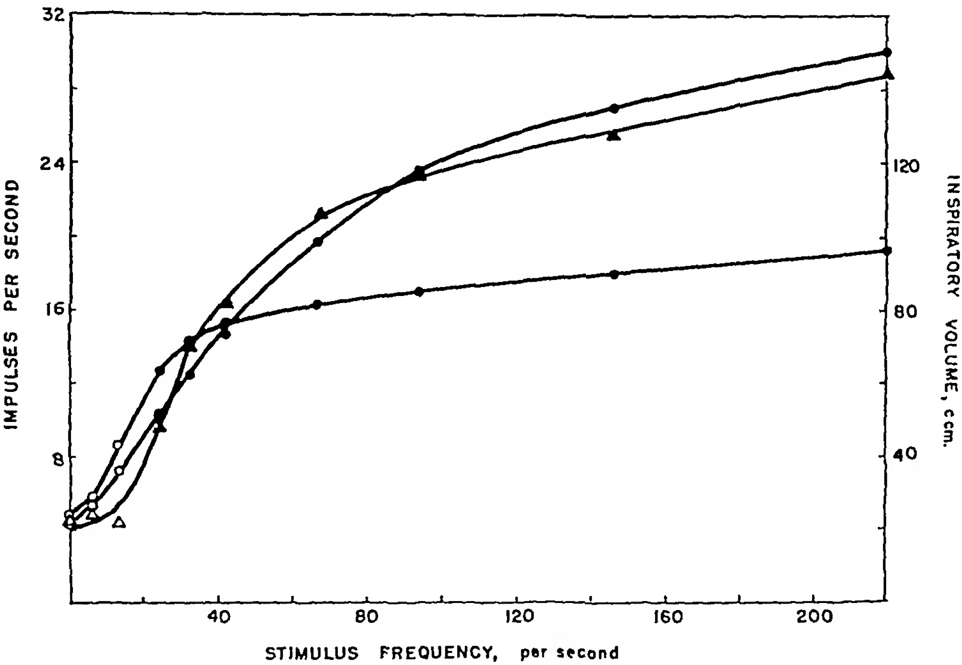

FIG. 4. Relation between the discharge of impulses by phrenic neurones, volume of inspiration and frequency of stimulation of the inspiratory center. Significance of symbols the same as Fig. 2. Intensity of inspiratory center stimulation, 8 v. throughout; frequencies, as indicated.

piration continues. But more intense or more frequent stimuli continue the process of prolongation of period of activity to its ultimate end, namely, continuous uninterrupted discharge whose frequency is a function of frequency and intensity of stimulation. Inhibitory mechanisms no longer can interrupt this intense discharge of the inspiratory center and maintained deep inspiration results.

Effect of expiratory center stimulation on phrenic neurone discharge

The behavior of phrenic neurones on stimulation of the expiratory center is in many ways the exact opposite of that on inspiratory center stimulation. In the control record of Fig. 5 two neurones which may be distinguished by differences in magnitude of spike potential, are active, one throughout the

inspiratory cycle, the other only at the peak of inspiration. Stimuli of an intensity of 2 and 4 v. and a frequency of 220 per sec. were applied to the expiratory center at the time indicated by the arrow, and maintained for the remainder of the record. The less intense stimuli inhibited activity of the neurone of greater spike potential and reduced frequency of discharge and duration of the period of activity of the other neurone. The more intense stimuli inhibited activity of both neurones completely. Still higher intensities served only to convert passive into active expiration. These results have been duplicated in all essential features by increasing frequency of stimulation at constant intensity.

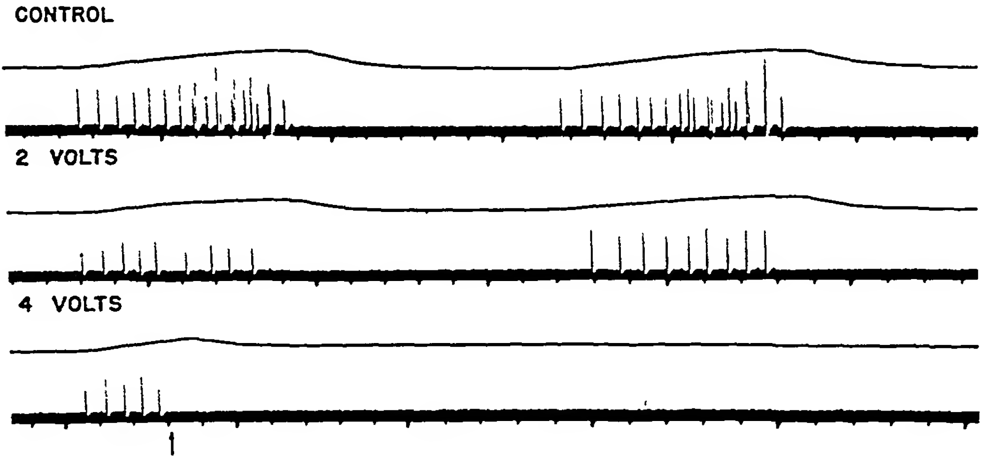

FIG. 5. Impulses discharged by two phrenic neurones and respiratory response on stimulation of the expiratory center with stimuli at a frequency of 220 per sec. and intensities of 2 and 4 v. Arrow marks beginning of stimulation. Time $\frac{1}{5}$ sec.

A comparison of Fig. 5 with Fig. 3 illustrates well the contrasting results on phrenic nerve discharge of expiratory and inspiratory center stimulation: "decruitment" as opposed to recruitment; decreased frequency of discharge as opposed to increased frequency; shortened duration of activity as opposed to prolonged; and finally with more intense stimuli, complete inhibition as opposed to continuous activity.

Connections between neurones of inspiratory and expiratory centers

Each of the two divisions of the respiratory center has an extent of over 30 c. mm. within the reticular formation of the medulla, yet the stimulation of but a small fraction of either leads to an essentially maximal respiratory response (Pitts, Magoun and Ranson, 1939a). It has been shown that a stimulus of 8 v. excites medullary structures only within a radius of 0.5 mm. around the tips of bipolar needle electrodes (Pitts, 1941), yet such a

stimulus applied to the inspiratory center at a frequency of 240 per sec. may produce an inspiration some ten times the normal tidal volume. Such experiments as well as more direct ones (Pitts, Magoun and Ranson, 1939b), indicate that the neurones making up the centers are extensively interconnected.

The general method utilized in performing our experiments throws additional light on the interconnection of the component units of the centers. Routinely single active fibers have been isolated from the phrenic nerve,

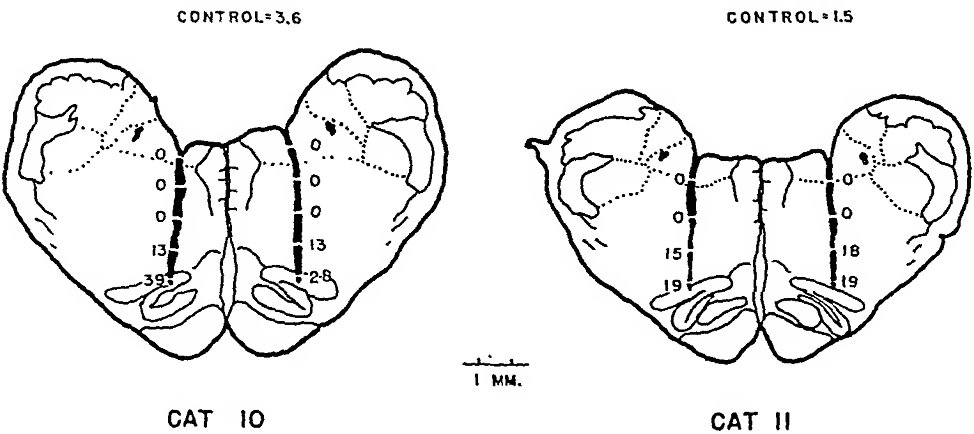

FIG. 6. Tracings of stained sections of the medulla oblongata of the cat showing electrode position and frequency of discharge of single phrenic neurones during stimulation at each level. Heavy vertical lines indicate track of the stimulating electrodes; the interruptions of the line, the position of the bare electrode tips. Numbers opposite electrode positions indicate frequency of discharge of a single phrenic neurone in impulses per sec. during stimulation. Control values above, give the frequency of spontaneous discharge in impulses per sec. Two separate experiments are shown. Stimulation at all levels in both experiments, 8 v. and 220 per sec.

the electrodes have then been placed within the general confines of the centers as previously outlined, either ipsilateral or contralateral to the phrenic fiber recorded from. In all instances, providing the electrode was accurately placed within the center, stimulation of the expiratory center inhibited, and stimulation of the inspiratory center excited that specific phrenic neurone chosen entirely at random. Fig. 6 (cats 10 and 11) shows the results in two experiments of stimulation of both sides of the medulla at millimeter inintervals with stimuli of an intensity of 8 v. and a frequency of 220 per sec., recording the activity of a single phrenic fiber split off from the left nerve. The spontaneous activity in impulses per sec. is shown as the control value above the tracing. The heavy black lines interrupted at millimeter intervals show the successive levels of penetration of the needle electrode. The effect of stimulation at each level is indicated by the number opposite the break in the line, representing the frequency of impulse discharge per sec. Out of several experiments performed these two were chosen for presen-

tation, for at each position of the electrodes the respiratory response was maintained constant, uninterrupted by rhythmic respiration. Thus at those levels marked with a zero, complete inhibition of activity was obtained, while at the levels marked by numbers, continuous discharge at the indicated frequency resulted. It is evident that a given phrenic neurone may be activated or inhibited from widely separated regions of the reticular formation of both sides of the medulla. While cat 10 showed a somewhat greater response of the single left phrenic neurone from stimulation of the most ventral level on the left side of the medulla, in general there has been no uniformly greater ipsilateral response. Since descending connections between inspiratory center and phrenic neurones are almost entirely if not completely uncrossed (Pitts, 1940), it follows that component units of the centers are connected and that stimulation of a few may activate many or all. In addition, equally extensive connections of an inhibitory type must exist between the two centers, for activation of a millimeter cube of the expiratory center leads to inhibition of the entire inspiratory center.

The experiments just presented indicate that the neurones making up both the inspiratory and the expiratory centers are richly interconnected. The latter exerts an inhibitory action on the inspiratory center, reducing the frequency and possibly the number of those neurones in activity. The withdrawal of excitation from phrenic motor neurones leads to cessation of their discharge and passive expiration. Active expiration probably results from more intense excitation of the expiratory center and consequent activation of expiratory intercostal motor units.

Characteristics of the excitatory process

In the preceding sections the general relationship between controlled excitation applied to the two divisions of the respiratory center and phrenic neurone activity have been presented. More detailed knowledge is obtained by closer examination of the relation between stimulus and response. For this purpose phrenic neurones have been chosen which were not spontaneously active, so as to eliminate confusion of spontaneous impulses with the response to a specific stimulus. Records have been taken at higher paper speeds in order to separate stimulus artifact and impulse satisfactorily. In Fig. 7 are presented the results of stimulation of the right side of the inspiratory center on the discharge of a phrenic neurone dissected from the contralateral phrenic nerve. The stimulus frequency was maintained at 14 per sec. and the intensity varied between 4 and 12 v. The stimulus artifact is barely visible as a fine line spaced at regular intervals throughout each record. At an intensity of 4 v. the neurone did not respond; at 6 v. the response occurred only during a part of the inspiratory cycle; at 8 v., throughout inspiration and occasionally in expiration. At an intensity of 12 v. the neurone responded to every stimulus throughout the whole respiratory cycle. It is evident that the neurone responds when it does so at all with a relatively fixed latency following a given stimulus. The latency

varies between 8 and 16 m. sec. This variation in latency has almost the appearance of being a random one; certainly it is not related to stimulus intensity. But there is a tendency for it to be somewhat shorter early in inspiration and to be longer in the expiratory phase. In general the picture is one of summation of subliminal excitation delivered to the phrenic neurone during inspiration with volleys of impulses synchronous with stimuli to

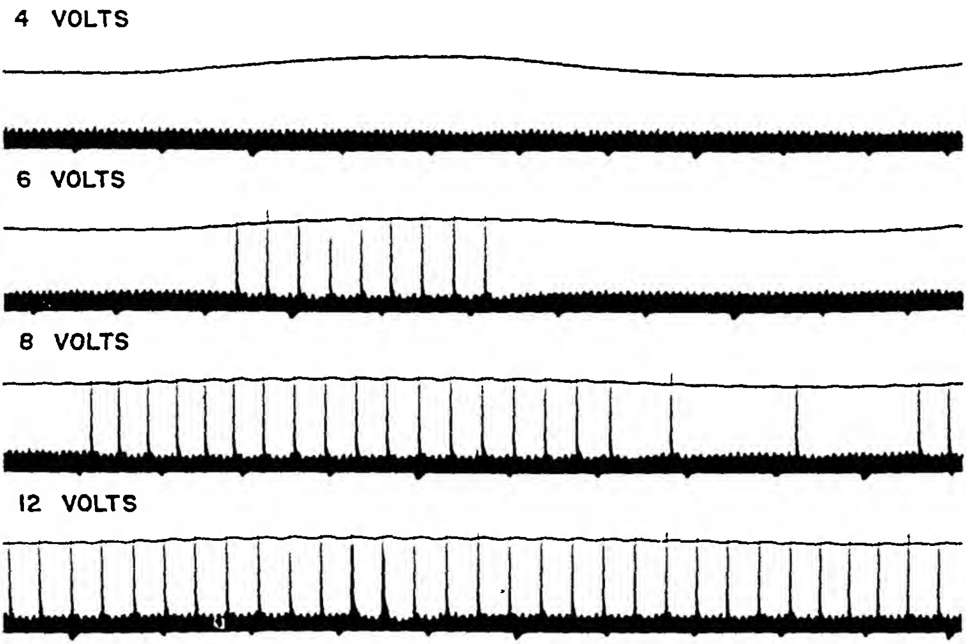

FIG. 7. Impulses discharged by a single phrenic neurone during stimulation of the inspiratory center at a frequency of 14 per sec. and intensities of 4, 6, 8 and 12 v. This neurone normally showed no spontaneous activity. Stimulation was continued throughout each record. Stimulus artifact barely visible as a faint trace at regular intervals in all records.

the inspiratory center. At the highest intensity, these volleys are themselves of sufficient magnitude to excite not only during inspiration but also during expiration as well. The latency of the response is a composite of phrenic nerve conduction time, synaptic delay at the phrenic neurone and at whatever internuncials may be involved, spinal cord conduction time, and conduction time and delay within the center. These several contributions have not been individually assessed as yet. These facts seem evident: (i) that this neurone, though inactive normally, is receiving subliminal excitation; (ii) that each stimulus to the inspiratory center is followed by a synchronous volley of impulses, sufficient at intensities of 6 v. or more to trigger the neurone and cause it to fire an impulse; and (iii) that the magnitude of this

volley is related to stimulus intensity and at 12 v. is capable of exciting even during the expiratory phase when background activity is at a minimum.

Additional light is cast on the nature of the normal activity of the inspiratory center by studying the response of phrenic neurones to stimuli of constant intensity and varying frequency. In Fig. 8 is presented the result of stimulation of the inspiratory center with stimuli of an intensity of 8 volts

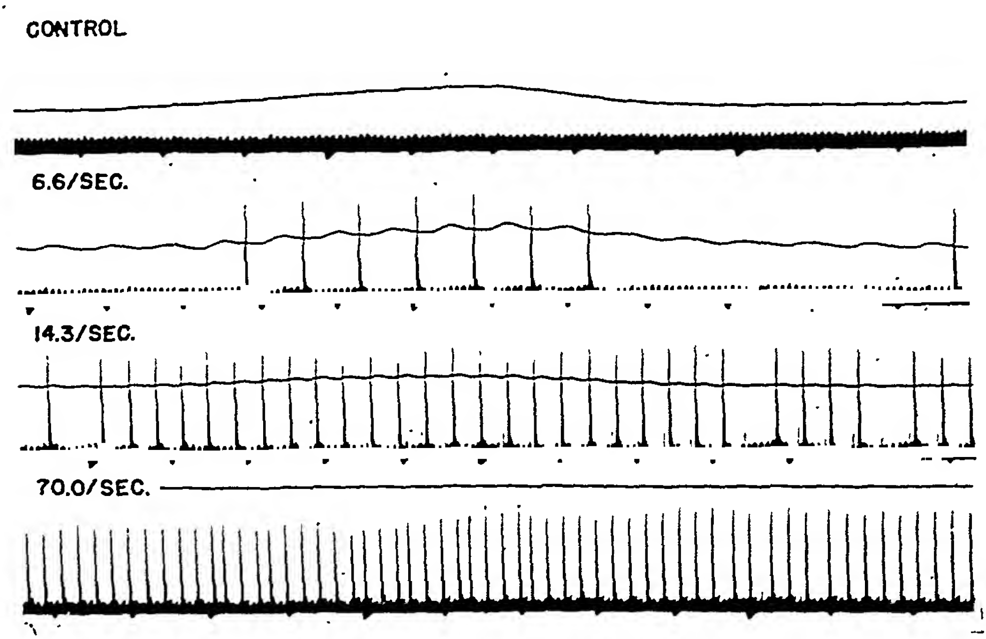

FIG. 8. Impulses discharged by a single phrenic neurone during stimulation of the inspiratory center at an intensity of 8 v. and frequencies of 6.6, 14.3 and 70 per sec. This neurone normally showed no spontaneous activity. Stimulation continued throughout each record. Stimulus artifact barely visible as a faint trace at regular intervals in all records.

and frequencies of 6.6, 14.3 and 70 per sec. At a frequency of 6.6 stimuli per sec. some seven stimuli were effective during inspiration while the five falling within the expiratory cycle were ineffective in eliciting an impulse. Increasing the frequency to 14.3 without altering intensity rendered most of the stimuli effective during both inspiration and expiration, though during the latter phase an occasional impulse was dropped. At both frequencies each impulse follows a given stimulus with a latency of from 8 to 15 m sec. It seems probable at the lower frequency of stimulation that each volley of impulses resulting from a stimulus sums with *subliminal excitation* delivered to the phrenic neurone during spontaneous activity of the inspiratory center, and triggers an impulse. But it would also appear that each stimulus leaves

in its wake a residuum of excitation* which persists long enough for stimuli at the higher frequency to facilitate succeeding ones. Accordingly stimuli trigger impulses throughout the expiratory cycle even though normal background activity is minimal. At a frequency of 70 per sec. the same triggering of impulse by stimulus is noted, the latency being essentially the same as at the lower frequencies. On an average, however, only every third stimulus is effective, although every fourth or every second occasionally triggers an

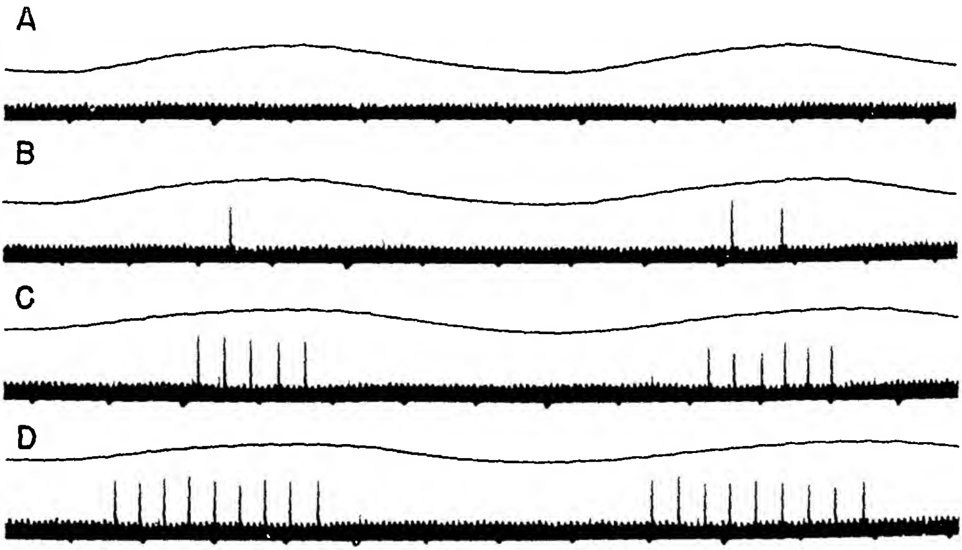

FIG. 9. Impulses discharged by a single phrenic neurone during stimulation of the inspiratory center at a frequency of 14 per sec. and an intensity of 4 v. A to D, progressive increase in chemical stimulus to respiration produced by rebreathing oxygen after removal of the carbon dioxide absorption tube. This neurone showed no spontaneous activity either normally or after rebreathing.

impulse. Certain volleys of impulses set up by stimuli succeeding each other at such short intervals find the motor neurone relatively refractory and fail to trigger it. At such a frequency the activity of the inspiratory center is so completely dominated by stimulation that the discharge of the phrenic neurone is continuous, and respiratory rhythm is lost. Frequency of firing of the neurone is then determined by the level of excitation maintained by the continued volleys of impulses from the inspiratory center and the rate of recovery of excitability of the neurone once it has discharged an impulse. At higher stimulus frequencies the neurone fires at a higher rate but not in proportion to the increase in frequency. The rate of firing at 70 per sec. was

* While this might mean a persisting change initiated by a given impulse at a synapse, it is more logical in view of delay pathways through internuncials and between neurons making up the center to consider that it represents a temporally dispersed volley of impulses.

25, approximately 3 to 1; at 220 per sec. it was 38, approximately 6 to 1. Tripling stimulus frequency probably falls short of tripling the number of impulses per sec. delivered to the phrenic neurone. Also the degree of subnormality of the neurone may be increased by the increased rate of firing. Both factors would tend to reduce proportionality between stimulus and impulse frequency. The results presented above apply equally well to neurones which show spontaneous activity, although they are less apparent because of the interpolation and interference of spontaneous impulses.

A third type of experiment gives additional information on the character of the normal excitation of phrenic neurones by the inspiratory center. Stimuli of an intensity of 4 v. and a frequency of 14 per sec. failed to excite the neurone studied as shown by record A of Fig. 9. The same stimulus intensity and frequency were maintained throughout all records from A to D. Carbon dioxide was allowed to accumulate in the inspired gas by removal of the soda lime absorption tube. Records B to D illustrate the effects of a progressive increase in chemical stimulus coupled with a constant electrical stimulus. The stimuli applied to the inspiratory center reach threshold and fire the neurone over a progressively increasing period of the inspiratory cycle as activity of the inspiratory center is increased by the chemical stimulus. It is logical to assume that under normal conditions (record A) subliminal excitation from the inspiratory center and subliminal volleys resulting from stimuli still did not reach threshold proportions; hence the neurone did not fire. Increasing excitation from the inspiratory center by increasing the carbon dioxide tension of the inspired gas enabled the volleys to reach threshold and trigger impulses earlier and earlier in the inspiratory cycle. At no time, however, during this series was normal excitation sufficient to cause the neurone to fire spontaneously.

Summary

Low intensity or low frequency electrical stimulation of the inspiratory center reproduces the same effects on phrenic neurone discharge as normal chemical activation of the respiratory center, namely: increased frequency of discharge; increased duration of activity; and recruitment of inactive neurones. Stimulation of the expiratory center, on the other hand, produces exactly the reverse effects, namely: decreased frequency of discharge; decreased duration of activity; and reduction of numbers of active neurones. Results obtained on varying stimulus intensity or frequency indicate that each phrenic motor neurone receives excitation from the inspiratory center over a number of separate pathways. The average level of excitation of the neurone is dependent upon the number of these pathways functioning and the frequency at which they transmit impulses. The rate at which the neurone fires is a function of its level of excitation and the time course of recovery of excitability once it has discharged an impulse. The inspiratory center-phrenic neurone system to this extent is similar in behavior to the hypothalamus-sympathetic motor neurone system described by Pitts, Larrabee and Bronk (1941) and Pitts and Bronk (1942).

Probably all the neurones making up the phrenic motor pool receive some excitation from the inspiratory center during eupneic respiration though for a large fraction it is of a subliminal degree. Any increase in activity of the neurones of the inspiratory center not only increases the frequency of discharge of those phrenic neurones already active but also recruits previously quiescent ones. Such an increase in activity of the constituent neurones of the inspiratory center may be produced experimentally by increasing either their frequency of discharge or the number active. It is probable that the same mechanisms operate normally under conditions which augment respiration.

Each phrenic neurone is functionally related, at least potentially, with each of the constituent neurones of the inspiratory center owing to their rich synaptic interconnections. Neurones of the expiratory center are similarly interconnected, and by virtue of their inhibitory influence on the inspiratory center, are capable of withdrawing excitation from phrenic neurones. Connections within a center probably serve to synchronize activities grossly with respect to phase of respiration and to facilitate those units inherently less excitable. Connections between centers are mutually inhibitory and prevent simultaneous activity of antagonistic elements.

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EFFECTS EVOKED IN AN AXON BY THE ACTIVITY OF A CONTIGUOUS ONE*†

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THERE IS no doubt that the activity of an element in the midst of a cell agglomeration can influence that of its neighbors, even when specialized contact surfaces for transmission, *i.e.* those loci traditionally known as synapses and which have been endowed with particular properties are lacking.

When the cell-units already are rhythmically active, their rhythm may synchronize more or less rigidly about that of one of the elements, which thus becomes the "pace-maker." Analysis of this is favorable in the agglomeration of cells of a nervous tissue, owing to the absence of mechanical factors which must enter into the synchronization of cardiac cells, of elementary muscular contractions (4) or of spermatozoa tails (60).

Amongst the phenomena of synchronization of nervous elements, that of cortical and ganglion cells (2, 3, 5, 5a, 59) are specially interesting. But although the vicinity interactions certainly play an important part in these phenomena, it is not always possible to eliminate that played by the specialized synapses. (See however experiments of Libet and Gerard, 51, 59). Synchronizations observed on isolated neural conductors do not suffer this difficulty.

Adrian (1) suggested that the synchronization of impulses in the isolated phrenic is possible because "an active fibre can cause a slight momentary increase in the stimulus to other fibres and that it can do so owing to the action current which it produces."

Certain conditions are required for the phenomenon to appear and be lasting. Arvanitaki and Fessard (19) showed that, for two spontaneous fibres of the crab nerve to interact, (i) their natural periods must be very close to one another, and (ii) the beats must be fairly well in phase. Also, the action potential of the pace-making element must be of sufficient amplitude. Thus, when several fibres with small action potentials and beating with similar rhythms do not synchronize, a large spike potential in a contiguous fibre may set them off together; but after a few beats in phase they again desynchronize (Arvanitaki, 10, p. 118, Fig. 63).

A rhythmically active element may progressively capture new quiescent

* Owing to the present circumstances this paper, finished since May 1940, could not be published earlier.

† Correspondence concerning this manuscript was prevented by war developments. It has been rewritten and shortened with every effort to retain the author's interpretations and references. For any misconstructions of her intent, I must assume responsibility. R.W.G.

elements so that an ever larger and more spreading response follows the rhythm of the pace-making element; the recruitment phenomena observed on myelinated (49) and on crab nerve (Arvanitaki, 10, p. 118).

Finally, a real transmission has been observed. In a crab nerve a single impulse arriving at a differentiated point may introduce a rhythmic impulse discharge in the same fibre and in a contiguous one; a pseudoreflex effect (8, 9, 21). Jasper and Monnier (56) more directly proved transmission of excitation from an element in one crab nerve to another placed in contact. The transmission delays they record were very variable but averaged 28 msec. Such considerable delays are hardly consistent with the required speed of transmission in synchronization and recruitment phenomena. Lastly, the older experiments of Bethe, proving that reflex movements of the crab antenna could be procured after ablation of the cell bodies—recently confirmed (70) on the stellar ganglion of Loligo—should probably be interpreted as an axon-axon transmission between the pre- and postganglionic fibres at the level of the section.*

Such preparations involve many unknowns: the number of active fibres, the elements influenced, the position and condition of the contact surfaces, the relation of pickup to the critical locus, the amount of shunting, etc. Conditions for analysis are much more favorable with an axon to axon preparation of the isolated giant fibres of *Sepia*. With this the strict determinism of the transmission of excitation at the zone of contact, and intermediary mechanisms which determine graded subliminal effects, are brought to light.

The experimental axon-axon contact had been referred to as an "experimental axon-axon synapse" (15, 16). But the word "synapse," of established usage and designating certain anatomically differentiated loci with particular properties, should perhaps be avoided. The mechanisms of transmission are doubtless similar in the two cases but not identical; and the contiguity interactions deserve separate consideration, for they may be important in nervous physiology and pathology. We therefore propose the term "ephapse"† to designate the locus of contact or close vicinity of two active functional surfaces, whether this contact be experimental or brought about by natural means. It may be the locus of contact of two absolutely homomorphous surfaces, for instance an axon-axon or soma-soma contact of two nerve cells. It would therefore differ from the word synapse‡ whose meaning is narrower and designates surfaces of contact (whether axosomatic, axodendritic, or axomuscular) anatomically differentiated and functionally specialized for the transmission of the liminal excitations from one element to the following in an irreciprocal direction.

* Compare the experiments of Osterhout and Hill (65) and of Auger (20) showing transmission of excitation from one plant cell to another contiguous one or across a salt bridge.

† From εφαπτω, to touch on or to, to attach; hence εφαψις, the action of touching.

‡ From συναπτω, to touch or attach together, to contact; hence συναψις is the action of joining, of linking, the union.

METHODS

Two giant axons of *Sepia officinalis* (69) of equal diameter (200 to 300μ) and 3 to 4 cm. long were isolated as described (11). The normal and identical excitability and conductivity along both axons are then assured by spike potential tests. The first axon, A, is placed on 4 leads, *ab* for stimulation and *cd* for recording, the end resting on small paraffin supports. Axon B is placed in contact with axon A for a length of about 5 mm. (*gh*) and on leads *ij*, *i* being close to the contact zone (Fig. 1).

Particular care was taken that the leads were well insulated to avoid leaks and the formation of secondary stimulation leads. The leads on which axon A rests were not supported by the same wall as those on which axon B rests. Leads were chlorided silver blades covered with threads soaked in seawater and fixed with sealing-wax into holes in the ebonite wall of the chamber. Condenser discharges were used for stimulation. Potentials were amplified with direct coupled amplifiers and the deflections of moving iron oscillographs (Dubois) recorded by means of a bromide paper camera. To record the potentials between leads *cd* and *ij*, two completely independent amplifiers with balanced input stages (63) were used.

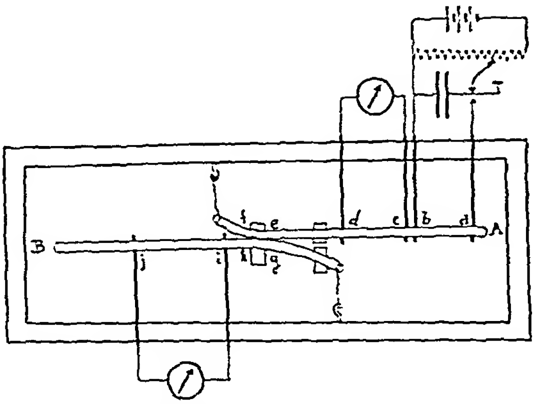

FIG. 1. Diagram of the experimental arrangement of the axon-axon preparation
A. afferent or preephaptic axon
B. effector or postephaptic axon
a, *b*, stimulation leads—*c*, *d* and *i*, *j* recording leads.
d and *i* can be moved and accurately adjusted.

Experimental Results

We shall call the stimulated axon an *afferent* or *preephaptic axon*, and its impulse similarly. Axon B, subjected to the action of A, and its response, will be designated as *effector* or *postephaptic*. Two cases must be considered: (i) the postephaptic axon B initially at rest and (ii) this axon spontaneously active.

I. The postephaptic axon initially at rest

The afferent axon A is stimulated by a brief condenser discharge of subthreshold intensity. The local response, or prepotential, near cathode *b* is recorded in *cd* (Fig. 2). As the intensity of the stimulus is gradually increased, the height of the local response increases progressively but no deflection is recorded on fiber B from leads *ij* (Fig. 2, I). When, however, a minute increase of the stimulus brings it to threshold a spike is recorded in *cd* (Fig. 2, I′A) and activity is suddenly exhibited by leads *ij* (Fig. 2, I′B). A brief diphasic deflection *pn* (a passive electric "escape" from the afferent spike) at *ij* is followed by a local wave of negativity *l* which represents sub-liminal activity evoked in region *gh* upon arrival of the afferent spike. Reducing the stimulus again eliminates all trace of activity at *ij*.

The preephaptic spike thus behaves towards axon B as a sub-liminal electric stimulus. To increase the relative stimulating value of the pre-

ephaptic impulse on region *gh*, this portion of axon B is soaked in a Na enriched solution isotonic to sea-water or, better, in a mixture of 8 parts of sea-water to 2 parts of isotonic trisodium citrate solution. Axon B is then placed as before and axon A stimulated. No activity is recorded by leads *ij* as long as the stimulus remains sub-threshold to A (Fig. 2, II), but as soon as a propagated response appears in A (Fig. 2, II′), activity also appears in B. A propagated B spike, starting from the top of the local response evoked in *gh*, is recorded in *ij* and, as electrical escape, e, upon the negative after-potential of fiber A (Fig. 2, II′A, see also Fig. 3). Washing the junctional region with a few drops of sea-water causes the response of axon B to become again sub-liminal although the spike at *cd* remains (Fig. 3, III); and resensitizing the contact zone *gh* with a drop of citrate solution immediately reestablishes the propagated response of B (Fig. 3, IV). This reversal can be repeated several times with no displacement of the axons, and therefore with no change in electrical relations, and the results described are infallibly obtained on every proper preparation.

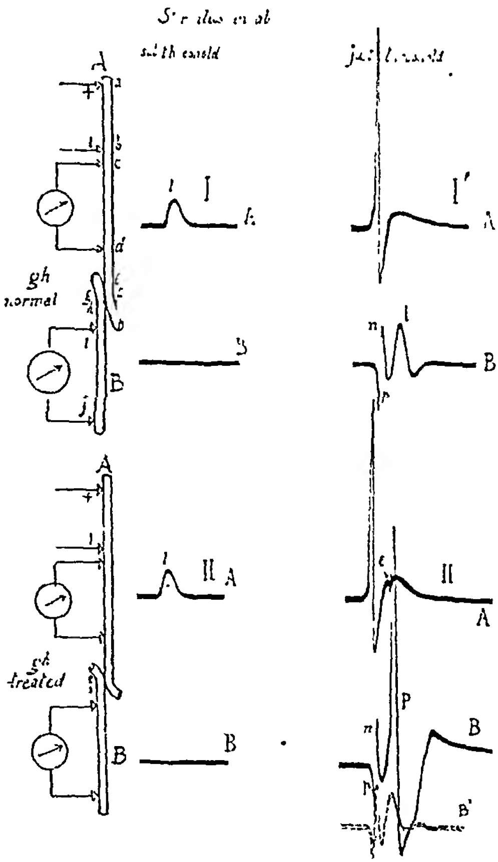

FIG. 2. Simultaneous records by means of two independent amplifiers of the responses of axons A and B, contiguous in *ef gh*, to a sub- or just-threshold stimulus applied at *ab*.

When the stimulus is sub-threshold and only a local response is recorded at *cd* (IA, IIA) no deflection appears at *ij*, whether region *gh* is normal (IB) or has been treated with citrate (IIB).

When the stimulus is just-threshold, a propagated spike recorded at *cd* (I′A, II′A) evokes a local sub-liminal response *l* (I′B) of the effector axon if region *gh* is normal and a liminal response P, if region *gh* has been treated (II′B).

These results can be summed up as follows: When the threshold of the postephatic axon is not sufficiently low, the incident spike potential evokes merely a local electric response in the contact zone, just as does a brief artificial electric stimulus of sub-threshold intensity.* When the threshold of the effector axon is lowered suffi-

* In a recent important paper Lorente de Nó (62), discussing his model of synaptic transmission, opened the question (see p. 449) whether a local "active" response might not be implied besides the "passive" electrotonic propagation.

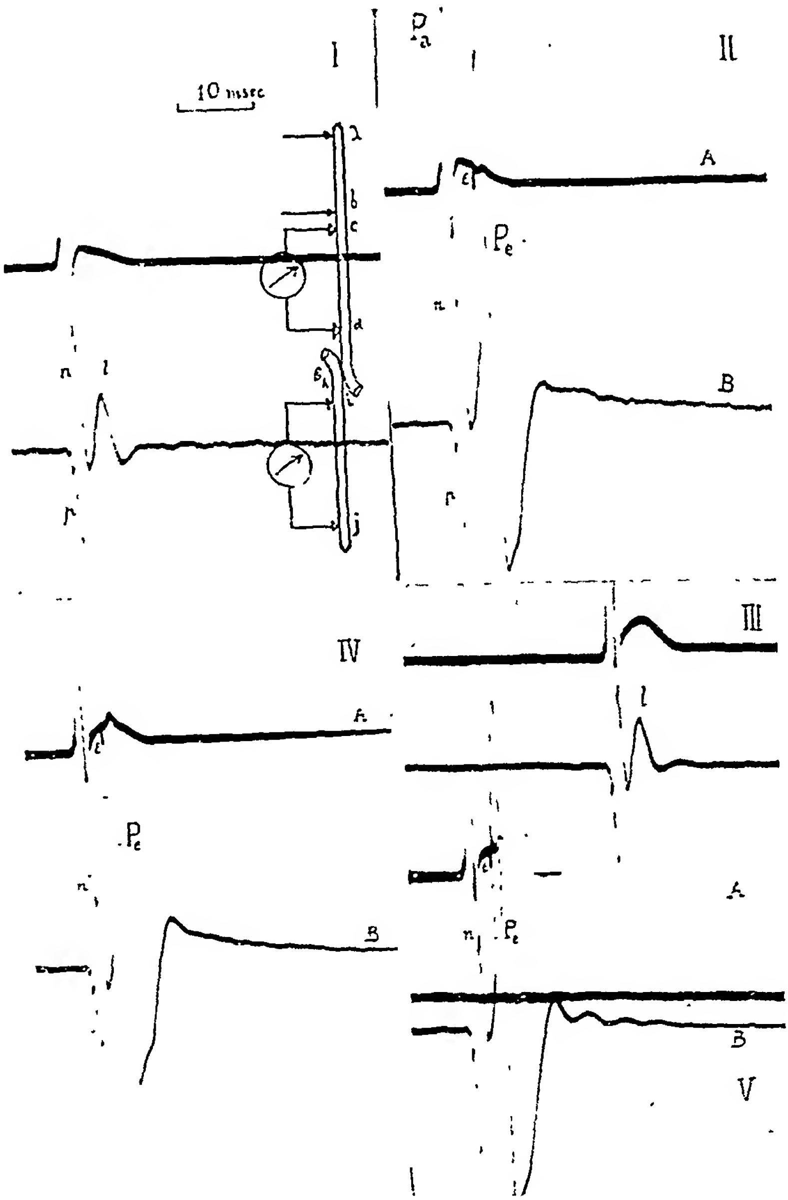

FIG. 3. Simultaneous records from the preephaptic axon, A (top curves) and the post-ephaptic axon, B (lower curves) of the responses evoked by a just-threshold electric stimulus applied at *ab*, as the region *gh* is altered:

I. *gh* normal. The reaction of axon B is only sub-liminal. After the diphasic effect of the afferent spike (see text), *pn*, recorded by electrode *i*, the prepotential *l* develops.

II. After citrate treatment. The reaction of axon B is liminal and an efferent spike P_e, is recorded at *ij*.

III. After washing. The reaction of axon B is again sub-liminal.

IV. Renewed citrate.

V. Third citrate treatment. The efferent spike P_e, is here followed by a local sub-liminal oscillatory response. Note that the transmission delay, *i.e.* the interval between arrival of the afferent spike at the contact region (indicated by the negative deflection *n*, see text) and the start of the efferent spike, is here 2 msec. shorter than in IV, B, and still shorter than in II, B.

ciently an afferent spike becomes a threshold stimulus and evokes the same response in the efferent axon as does a liminal electric stimulus; in both cases a spike starts at the top of the local response. This preparation of two isolated axons experimentally brought into contact is thus the prototype for transmission of excitation from one element to another, including subliminal as well as liminal effects.

The effective stimulus

In this preparation it is obvious that the external action currents due to the spike conducted by the active fibre represent the virtual stimulus to region *gh*, its immediate neighbor.

The effectiveness of the stimulus depends on the electric field created by this primary event in the exterior conductive media, particularly in region *gh*. An exact knowledge of the distribution of isopotential lines of this field and of their variation with the propagation of the spike along axon A would therefore be necessary here. It is on their characteristics that the variations of potential gradient to which the influenced segment is subjected will depend—variations which more directly determine the stimulating value of the afferent spike.

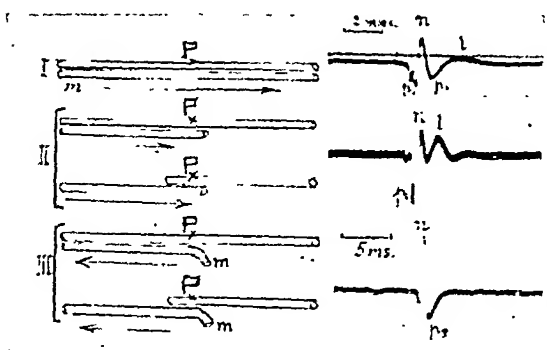

FIG. 4. Variations of the potential of an external point, P, as recorded by an interposed resting axon, when a spike in a contiguous active axon is propagated in the direction of the arrows (see text).

The electric field created by the action currents of an element in the conductive media that surround it has been considered from various viewpoints (26, 27, 28, 36, 45, 62, 68). Wilson, McLeod and Barker (68), after mathematical analysis, conclude that an action potential advancing in a linear element produces a potential change at any point in the surrounding conducting medium as would a similarly advancing dipole. Bishop (27) has reached similar conclusions as to the variations of potential at an exterior point, P, from consideration of the lines of force about a potential across the membrane of the conducting element. Relative to a distant point, M, the potential of P becomes: first relatively more positive as the spike approaches (phase p_1); then relatively negative (phase n), the maximum being reached when the excitation wave passes immediately under P; then again relatively positive (phase p_2), as the wave, having passed under P, moves away.

The conditions about point P may be less symmetrical. If, for example, the propagation stops in the conducting element immediately under P, the potential change at P will be restricted to the two first phases, p_1 and n. Conversely, if the wave starts under P, only the negative and second positive phases will appear (Fig. 4, III). And if P is far from the conductor only the positive phase may persist.

These three cases can be realized by leading from a resting axon the spike passing in a contiguous axon. The first is seen when axon A, having met the efferent axon, lies alongside it for some distance. The resulting triphasic wave (Fig. 4, I) lasts 1.5 to 2 msec., corresponding to the propagation time along A. Obviously, such an arrangement is not favorable to start a response of the efferent fiber. As a matter of fact, when this triphasic variation is followed by a prepotential wave, this wave is of negligible amplitude.

The second case is obtained when the spike in A is recorded from leads *ij* on axon B (Fig. 4, II); and the third when a spike started in region *gh* of axon B is recorded from electrodes *cd* on axon A. In this third case, the appearance of some or no negativity pre-

ceding the positivity depends on the distance of electrode *d*. Activity of B never evokes a response in A.

It is thus only in the second case, *i.e.* when external action currents due to the propagation of the afferent spike terminate in a more or less pronounced negativity, that subthreshold or threshold stimulation of the efferent fiber results. Actually the amplitude of the efferent response is a function of that of the negative phase, n, of the diffuse currents. Figure 5, for instance, reproduces the responses recorded from *ij* on axon B following threshold stimulation of axon A at *ab*, as region *gh* of B is progressively altered by citrate. Records I to III show the height of the prepotential response, *l*, increasing with the negative phase of the currents due to the afferent spike. Plotting as abscissae the heights of the negative phase of the "diffusion" wave and as ordinates the heights of the corresponding prepotential response gives the curve of Fig. 6. It is essentially the curve for electric stimulation of an isolated axon of the crab (52, 58) or of *Sepia* (12), when abscissa is the intensity of the stimulating current and ordinate the height of the prepotential. The negative portion of the external action currents thus represents the useful stimulating part for a second axon.

The visible evidence that citrate increases the height of the action currents of A which pass through B suggests that citrate diminishes the impedance of the membrane of the fiber on which it acts.

Since the negative phase of these currents tends to excite neighboring elements, the positive phases might similarly depress. Actually, in cases I and III the results are as if the final anodic positive wave were depressing the active response evoked by the cathodic stimulus. Katz and Schmitt (57) have recently shown a polyphasic change of excitability in a crab fiber when an impulse passes through an adjacent fiber. Their experimental conditions correspond to case I and in duration and configuration the excitability changes they describe correspond phase by phase to the curve of Fig. 4, I. The change of excitability and the change of external potential here considered are presumably concomitant phenomena.

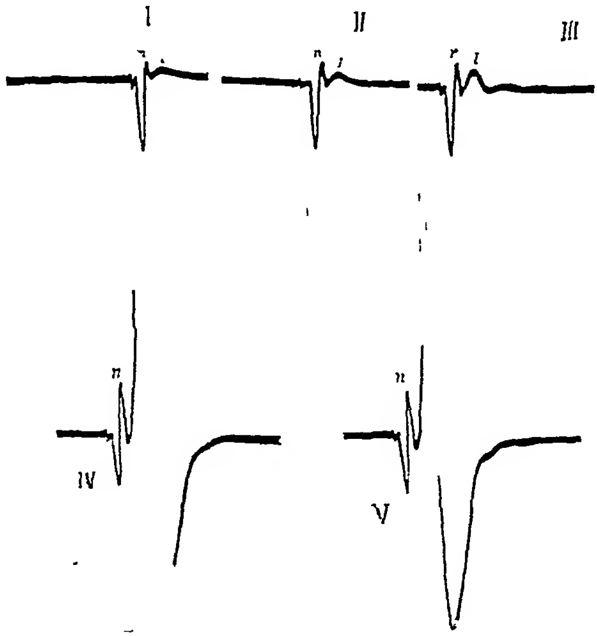

FIG. 5. Effects evoked by an afferent spike on the efferent axon B. Region *gh* is normal in I, and progressively citrated in II, III, and IV. The height of the negative phase, *n*, of apparent currents increases from I through IV. The local sub-liminal efferent action, *l*, increases at the same time from I to III and becomes liminal and propagated in IV and V.

Although not further discussed here, prepotentials and after-potentials of the afferent axon, as well as spike potentials, can produce detectable variations in the excitability of the efferent axon.

In short, the characteristics of the effective stimulus—duration, shape and amplitude—depend partly on the characteristics of the spike potential and on its propagation speed and partly on the distributed resistances and capacities of the external medium, as well as on the relative positions of the active and the influenced segments. And, as with any electric stimulus, the

changes in the influenced segment prior to its active response, *e.g.* its prepotential, are purely passive electric phenomena.

Delay of ephaptic transmission of the excitation

This delay represents the interval between the entry of the incident spike into the ephaptic region and the initiation of the postephaptic spike. It can be calculated precisely when axon A is long enough to permit the direct measurement of propagation velocity in it. Propagation time to the ephapse (3 msec. on the average) is then subtracted from the interval between the afferent spike recorded in *cd* and the efferent spike recorded in *ij* (under conditions of liminal transmission). This delay reaches a maximum value of 5 msec. The same value for the delay is obtained by taking the time of the negative wave in the potential field (leads *ij*) as the index of the arrival of the afferent spike at the ephapse.

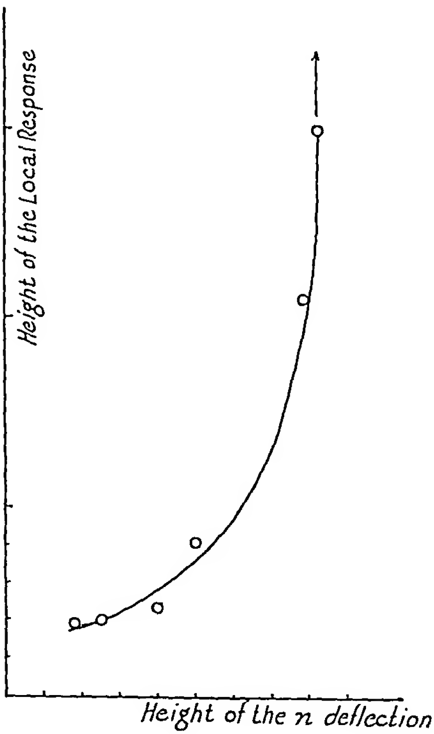

FIG. 6. Relation between the negative phase, n, due to afferent action currents and the height of the post-ephaptic local response, l. A spike starts on the prepotential response at the point marked by an arrow.

Two factors at least may contribute to this delay: (i) an interval before the postephaptic local response starts; and (ii) the time necessary for this response to reach its liminal level to set off the spike. The second factor is the more important and, as the excitability of axon B increases, and so the stimulating value of the incident spike, the duration of the ascending phase of the local potential decreases and the ephaptic delay with it. Variation of the ascending phase from 5 to 2 msec. (3.5 in Fig. 3, II, 2 in 3, V) is paralleled by similar variations of ephaptic delay. In myelinated axons, Blair and Erlanger (29) have shown a related fluctuation of latency of response to threshold induction shocks, over a 0.25 msec. range for most excitable axons, of 2.5 msec. for least excitable ones; as compared to a minimal latency of only 0.1 msec. with suprathreshold stimuli. The fluctuation is presumably here also in axon reactivity and in the development of the local response.

The spike potential of the preephaptic axon thus comports itself towards the postephaptic axon exactly as an artificial electric stimulus would have done. If the effector axon is relatively inexcitable, its response will be re-

stricted to the prepotential wave. If its excitability is such that the incident impulse should act as a liminal stimulus, the local response develops further and from the top of the prepotential wave, after time for its rise, a spike is propagated all along the fiber.

One particular case deserves further note. Under mild citrate action, the sub-threshold response of the treated region changes the aperiodic to the damped oscillatory type (12). This oscillatory response can be evoked not only by make or break of a constant current but also by a brief stimulus in the region of the cathode (Fig. F) or by a spike potential conducted along

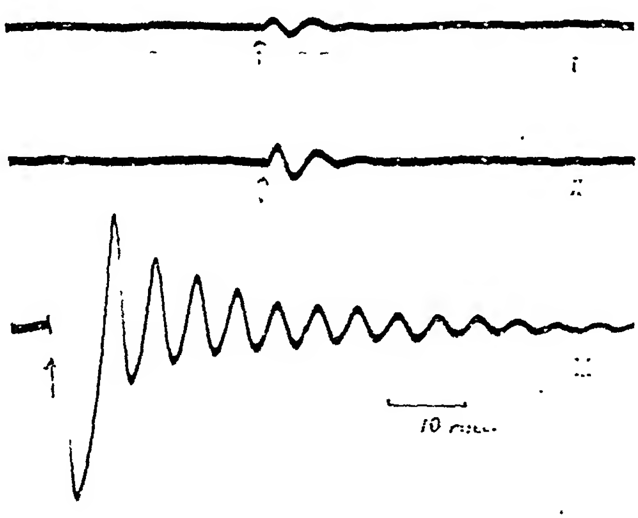

FIG. 7. Local oscillatory response evoked in a citrated axon region near the cathode of a brief sub-threshold condenser discharge. Three successive records show gradually increasing intensity of the stimulus marked by arrows. In III this is threshold and a spike starts on the first negative local wave.

the same axon to the citrated region (13). These same damped oscillatory reactions can be observed on the postephaptic axon B as a sub-liminal response to an incident impulse from A, after B has been repeatedly treated with citrate solution and washed with sea-water (Fig. 2, II′B′ and Fig. 3, III). After further citrate treatment of region *gh* of axon B, the arrival of an afferent spike, P_a, initiates an efferent spike, P_e, followed by a series of damped subliminal oscillations of the local potential (Fig. 3, V). P_e always starts at the top of the negative phase of the first sub-liminal undulation after the interval required for the local potential to reach its liminal value. If stimulation by an afferent spike is repeated several times a "facilitation" of the response results; the sub-liminal oscillations following the efferent spike increase in height until a rhythmic and sometimes prolonged discharge of spikes follows the first efferent spike. This may be called a post-ephaptic after discharge.

Thus, when the postephaptic axon is in the state to develop a sub-liminal oscillatory activity, it will respond to an incident impulse of subthreshold intensity with a series of regularly damped oscillations of its local potential, and to one of threshold intensity with an efferent spike starting at the top

of the first negative oscillation and followed by an after discharge that may be sub-liminal (damped oscillations of the local potential) or liminal (rhythmic impulse discharge). Again, the response to an incident impulse is exactly what it would have been to an electric stimulus.

II. The postephaptic axon is initially active
A. Sub-liminal oscillatory activity

Such oscillations can continue as long as several seconds, without initiating propagated spikes, and maintain a remarkably constant period, of the order of 7 to 10 msec. for the isolated axon of *Sepia* (12), and of 8 to 10 msec. for crab fibers (6). Such a sub-liminal activity often remains after a discharge of rhythmic impulses, spontaneous or evoked. Repeated treatment of the contact region *gh* of axon B with a sodium rich solution may finally lead to such a discharge following a single incident impulse from axon A. During such oscillatory activity of B, single afferent impulses set up in A (by condenser discharges) (Fig. 8) could be caused to reach B in different phases of its oscillations. The arrival of the afferent spike in the *contact* region *ef* is timed on record B by the negative phase of its escape (deviation *n*).

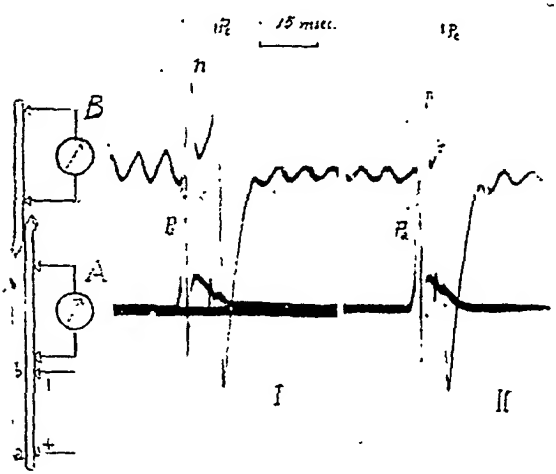

FIG. 8. Simultaneous records, from independent amplifiers of the responses of two axons continguous at *ef gh*, to an efferent spike evoked at *ab*. Axon B initially exhibited a sub-liminal oscillatory activity (upper record). Upon the arrival of the afferent spike P_e (lower record, and negative wave, *n*, of current speed in the upper record) an efferent spike, P_c, starts in the postephaptic axon.

In record I the afferent spike falls in the positive phase of the local oscillatory activity of axon B while in record II it falls in the negative phase. Note the different delays in transmission.

(*a*) *Incident impulse threshold.* With the afferent axon in good physiological state, its spike, P_a, always evoked in axon B an efferent response, P_c, (Fig. 8). The ephaptic delay, the interval between spikes P_a and P_e corrected for propagation time, is always shorter than the oscillation period of axon B. This time varies slightly and systematically, in many experiments, with the timing of the afferent spike relative to the cycle of sub-liminal activity of B: the delay is least at the maximum of the negative phase of the oscillatory potential and greatest at the maximum of the positive phase (Fig. 9; see also Fig. 8). In line with the previous discussion, since the absolute intensity of the stimulus (the afferent spike) remains unchanged, the fluctuation of

the ephaptic delay expresses the fluctuation of the excitability of the effector axon. And as the time variations follow closely the potential oscillations, this is evidence of an oscillatory variation of excitability. The existence of oscillatory variations of excitability has been also demonstrated after the make response of a fiber to a constant rheobasic current, and following the recovery from absolute refractoriness (period of the order of 5 msec.) (44); and after a brief sub-threshold shock to a citrated frog nerve (64).

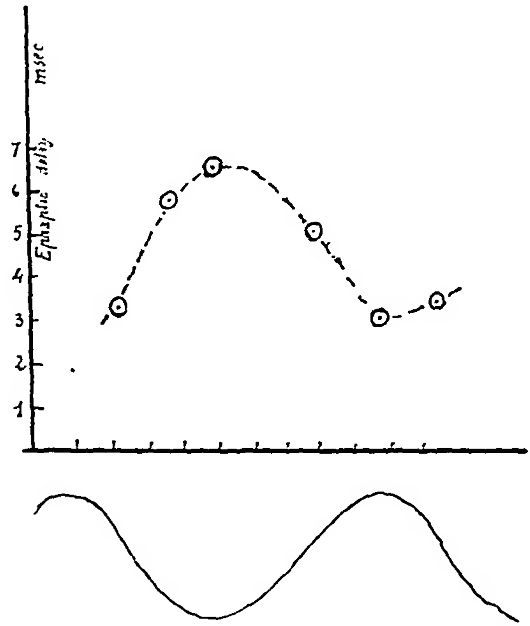

FIG. 9. Variation of the ephaptic delay with the timing of the afferent spike in the potential cycle of the postephaptic axon B, traced below.

Thus, in all the cases so far considered, whether the postephaptic axon is initially at rest or in oscillatory sub-liminal activity, when the afferent spike is great enough to constitute a threshold stimulus it evokes an effector response after an ephaptic delay which is determined principally by the development of the local postephaptic response. The local potential acts as the intermediary mechanism between the afferent and the postephaptic spike, just as it does between an electric stimulus and a spike.

(*b*) *Incident impulse sub-threshold—long and variable transmission delays.* The state of the afferent axon sometimes leads to a progressive depression of its spike potentials. It is then possible to apply a graded series of spikes to a postephaptic axon which is an oscillatory activity.

When the amplitude of the afferent spike is too feeble to evoke an immediate postephaptic spike, it nevertheless brings about a progressive increment of the sub-liminal oscillations of the effector axon. The more depressed the afferent spike, the less is this increment (Fig. 10*c*, *b*, *a*). The incrementing oscillations may finally suffice to set up a postephaptic spike, after a highly variable delay (Fig. 10*c*, *b*), or they may terminate without doing so (Fig. 10*a*). Since a spike ordinarily starts at the top of a negative wave of the oscillatory potential, long ephaptic delays differ by a whole number of subliminal oscillations; *i.e.* by essentially constant time increments equal to the period of the oscillations. The delayed transmission observed by Jasper and Monnier (56) across crustacean nerves probably is of this type. The average oscillation period of a crab nerve is about 10 msec. (6), and two periods would approximate a reported delay of 22 msec.

Similar long transmission times can be seen when the afferent axon is perfectly normal and yields strictly constant spikes, providing the postephaptic axon is temporarily depressed. In Fig. 11, III, *e.g.*, the postephaptic axon has just spontaneously emitted a spike, P_{sp_1}, (seen as spread, *e*, in the record from Axon A) when an afferent spike, P_a, reaches it. Under those conditions P_a is a sub-threshold stimulus and produces only gradual incrementing of the sub-liminal activity of the postephaptic axon; this, however, after three oscillations gives rise to an efferent spike, P_e.

The great latency of response, up to several hundred milliseconds, to constant currents or even condenser discharges, which is often encountered in invertebrate nerves (17) and occasionally in myelinated ones (43), is a similar phenomenon. Also, when one stimulus gives no spike, a second may sum its effect over 200 to 300 msec. (7). These and other facts show that the electric stimulus initiates slow processes, probably metabolic, which lead to augmented oscillations and final discharge of a spike.

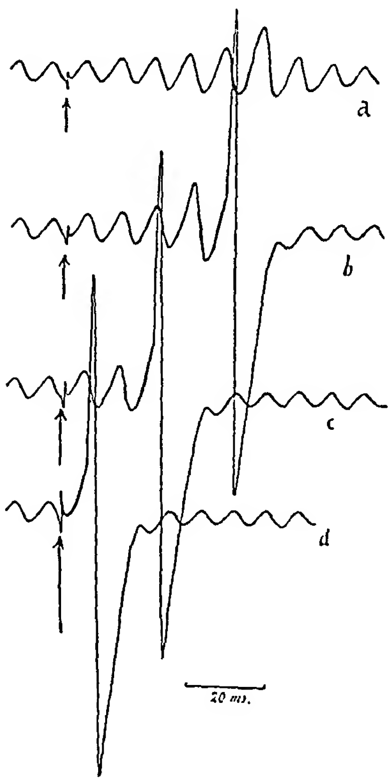

FIG. 10. Reactions of the postephaptic axon to an afferent spike of decreasing height, *d* to *a*. The arrows indicate arrival of the afferent spike.

B. The postephaptic axon is in an initial state of rhythmic liminal activity

In the case just examined the postephaptic axon was in a sub-liminal oscillatory state. Such sub-liminal activity may persist for a considerable time after repeated spike discharges, which in turn can be initiated by a single preephaptic impulse. At the beginning of such an autonomous discharge (11), a single spike may arise from the crest of each negative local wave (phase of homorhythmic activity), or (phase of heterorhythmic activity) groups of spikes are emitted, each from the crest of a negative wave but with intervening oscillations with no spikes. After discharge of a group of spikes the oscillations are much reduced and only gradually increment until another spike group can be set off.

In Fig. 11, I, axon B is autonomously rhythmic and several sub-liminal oscillations intervene between spikes. For some seconds an activity cycle was repeated with 13 to 16 oscillations, of minimal amplitude immediately after a spike and gradually increasing until the following spike is emitted. Axon A is inactive, although the escape current, e, from a spike in B (P_{sp})

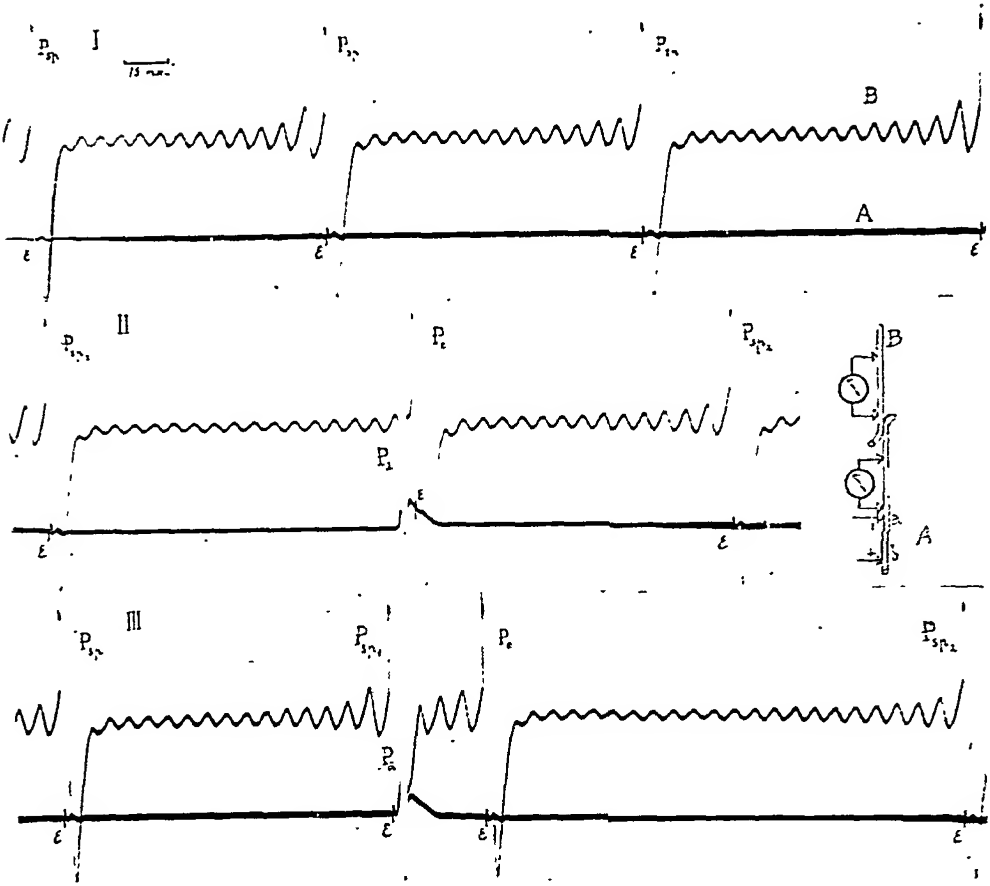

FIG. 11. Disturbances introduced by an afferent spike in the spontaneous activity of axon B (upper records):

I. Oscillatory activity of axon B, periodically becoming liminal. Only the current escapes, e, of the spontaneous postephaptic spikes, are seen in the lower record from the completely quiescent preephaptic axon, A. This activity has lasted several seconds with the interval between spontaneous spikes approximately constant at 13 to 16 sub-liminal oscillations. The events described in II and III have been superimposed on a spontaneous activity of this kind.

II. An afferent spike, P_a, evoked at *ab*, reaches the ephaptic region 16 sub-liminal oscillations after a spontaneous spike, P_{sp_1}; when axon B is due to start a second spontaneous spike. An efferent spike, P_e, starts after a delay of 3 msec., thus substituting for the spontaneous spike. The subsequent cycle of rhythmic activity of axon B is not disturbed; a new spontaneous spike, P_{sp_2} starts after 14 sub-liminal oscillations.

III. An afferent spike, P_a, evoked at *ab*, reaches the ephaptic region shortly after the emission of a spontaneous spike, P_{sp_1}. It evokes an efferent spike, P_e, after three subliminal oscillations of the prepotential. The rhythm of subsequent cycles is disturbed, the next spontaneous spike, P_{sp_2}, being emitted after an interval of 22 sub-liminal oscillations.

is seen in a record from it. Stimulation of A imposes an afferent spike at some point in the cycle of B and evokes an efferent spike. The transmission delay is long if the afferent spike arrives in the depressed period just after a spon-

taneous spike (Fig. 11, III), short if it arrives later (Fig. 11, II); and in either case it disturbs the cycle of spontaneous activity of B.

The interval I″ between the extra-spike, P_e, and the next spontaneous spike P_{sp_2} is not equal to the regular interval I between spontaneous spikes, but is an inverse function of the interval I′ between the previous spontaneous spike, P_{sp_1}, and the extra-spike. The extreme cases are illustrated in Fig. 11. In Fig. 11, III the incident spike P_a arrives shortly after emission of the spontaneous spike P_{sp_1}, brings about a quick increment of the height of the following subliminal oscillations, and so starts an extra response, spike P_e, prematurely in the cycle. Interval I′ includes only three sub-liminal oscillations and interval I″ includes 22 sub-liminal oscillations, about 1.5 I. When interval I′ is greater the consecutive cycle I″ is proportionally shorter. The other extreme is shown in Fig. 11, II. I′ is 16 cycles and I″ is 14; the spontaneous spike due to appear is suppressed and the next one appears after a 14 cycle interval.

Thus an early extra-spike is followed by a compensatory pause (I″–I) in the following cycle. But I′+I″ is usually less than 2 I so the spontaneous rhythm is resumed out of step with its earlier portion. A late extra-spike, however, supplants the spontaneous one soon due, presumably discharging the same accumulated excitation, and does not disturb the spontaneous rhythm. The comparable interruption by an afferent impulse of a motoneuron rhythm (Eccles and Hoff, 1932; Hoff, Hoff and Sheehan, 1935) may be recalled. Also, the ability of an incoming impulse to throw out of phase a group of synchronized elements is relevant here.

Discussion

The phenomena demonstrated on the experimental axon-axon contact can be directly transferred to the central synaptic problem, after consideration of the particular reactive characteristics to currents of the receptor element, neuron soma, and of the specific geometrical conditions of the synaptic contact. The latter are, of course, more complex than for the tangential axon-axon contact, and the asymmetry between terminal knob and cell body or dendrite may determine irreciprocal conduction by the difference in stimulating efficiency of the electric fields from one element on the other (compare case II and case III, Fig. 4). The action currents of multiple afferent knobs acting on a single receptive surface would similarly make for unidirectional conduction.

As regards the electric reactivity of the soma, certain data are available. The somata (spinal cord, superior cervical ganglion, oculomotor neurons) respond to an afferent volley by a considerable early negativity which is apparently longer than the spike (66, 54, 37, 22, 23, 40, 61, 30). In certain cases it appears to be followed by a positive phase (66, 54, 37, 40, 62). The reactions evoked by two spikes in the same afferent fibre are cumulative (24, 46). Gradation of the electric or mechanical response may be due to the addition of new units; in fact, although authors unanimously admit the

existence of a state of central excitation of essentially graduated character, they generally do not invoke a central graduated electric response. The chief cause of this abstention appears to have been the lack of demonstration of such a response of the peripheral myelinated nerve (see 5, 44, 62). However, since the functional specialization of a cell body is for accumulating, integrating and modifying the effects of stimuli, this small structure may well have different electrical properties than the long axon specialized for conduction. Actually the graduated prepotential may be the best counterpart of the soma's electric response, as suggested by records from central neurons (5, Fig. 6, B; 2). When few, or one, cortical neurones participate in an injury discharge, the spontaneous waves closely resemble the oscillations in invertebrate axons. In the visceral ganglia of *Aplysia*, large cells (200 to 300μ diameter) are easily distinguished amongst numerous ones of small dimensions (18), and these are favorable for observing the electric reactions of a single cell initially at rest to afferent impulses (34). This electric somatic response is a graduated one and shows the types seen in the axon, from an aperiodic negative wave to damped or sustained oscillations.

Thus the soma is probably the normal locus of the neuron exclusively specialized for local and graduated activity, although the axon can behave similarly at a point of stimulation. The ephapse behavior then throws light on some facts about synapses.

The brief excitation state (detonator action), produced by an afferent impulse arriving at the synapse, and evidenced by a summation period (61), is doubtless brought about by the electric field of the impulse arriving in a terminal knob. This process contributes to the summation of impulses arriving quite or almost simultaneously along different afferent fibers reaching the same soma (38, 62). It may be emphasized that this is a passive electric phenomenon, resulting from currents created by the advent of the afferent spike and by means of which it stimulates. No active response of the cell is involved and so no electric change in it—except by passive spread. The graded response of the soma follows, with its activity. What its character will be depends on whether the afferent impulse reaches the soma when this is spontaneously active (as in cortical neurons), in which case the phenomena described in part II will follow; or when it is at rest, in which case the part I effects follow—an aperiodic negative wave (as in cord and ganglion cells of vertebrates and *Aplysia*) or a train of damped oscillations (as in *Aplysia* and perhaps in deeply anaesthetized cortical cells). When the somatic depolarization is sufficient, the longitudinal potential gradient from the soma to the emerging axon initiates an efferent spike. If the liminal value is not reached "d'emblée," sub-liminal electric and excitability changes occur, as indicated by data on summation intervals. The classical optimum summation interval of 8 to 10 msec. (41, 32, 33) would be due to the first sub-liminal negative wave.

Summation at successive negative maxima is obviously difficult to detect due to the many disturbing factors, yet some evidence for it exists in a

second and more extended (due to partly out-of-phase negativity in different neurons) optimum summation interval (41, 33, 26).

In normal intact nerve the propagated activity of one axon submits each point of adjacent axons to the action of triphasic transient currents (Fig. 4, I), which cannot readily excite because of the final positivity. Moreover the interposition of myelin and of interstitial tissues between the functional surfaces efficiently insulates them from local currents. During normal propagation, therefore, the influence on neighboring fibers should be negligible. But if propagation is stopped at a cut or blocked region of the active axon, the conditions (case II of Fig. 4) favor stimulation of contiguous fibers; especially if these have been subjected to staling, alcohol, or other factors which diminish the insulating efficacy of their myelin. The synchronization and ephaptic phenomena do occur in nerve under just these conditions.

If the local sub-liminal activity itself constitutes a significant stimulus to neighboring axons, as experiments to be reported elsewhere show it does, a peculiar quasi-sinusoidal form of physiologically functional stimulus becomes important. Such a stimulus is liable to variation of frequency and amplitude under the influence of many factors (12) and so introduces many new possibilities of electric action at junctions, beside the simple brief, monophasic, all-or-nothing spike potential. Such considerations apply also to central cell bodies, especially since these have little lipid insulation at their surfaces (25, 35); and the existence of ephaptic interactions between cell bodies seems highly probable. The same factors which favor axon synchronization also favor central synchronization. Synchronization by synaptic paths cannot be formally excluded, but strong evidence indicates the efficacy of ephaptic interactions and their predominance in abnormal cases. Direct experimental evidence for this is seen in the synchronization of retinal neurons (5a) and of those of the olfactory bulb of the isolated frog brain following synaptic paralysis by nicotine (59; *Ed.:* see also 50, 51).

Study of the experimental axon-axon contact should thus throw light on liminal and sub-liminal synaptic phenomena and on the more general ephaptic interactions determined by current flow between near-by elements, which contribute to transmission and coordination.

Summary

Several conditions of contact (ephapse) between two isolated *Sepia* axons permit study of the electric reactions evoked in one (initially active or at rest) by the electric activity of the other.

The current spread from the active axon serves as a polyphasic electrical stimulus to the contiguous axon. The general appearance, height and duration of this stimulus depend, on the one hand, on the shape, height and propagation speed of the action potential of the active fiber; and, on the other, on the geometrical conditions of contact and on the excitability characteristics of the receptive axon.

When the contact conditions are such that the currents penetrating the

resting axon have a large final positive phase, they produce no visible effect on it. When this final positive phase is sufficiently depressed, the effects of the preceding negative phase predominate and evoke characteristic subliminal or liminal active electrical reactions of the resting axon. This case is realized for the portion of the resting axon in contact with a termination of the active axon and to a spike ending there. The phenomena in such an experimental arrangement have been observed and analyzed in detail. The active axon which conducts an impulse towards the contact region has been called the afferent or preephaptic axon; the axon acted upon by it, the efferent or postephaptic axon.

When contact conditions are such that the action currents of the afferent impulse constitute only a subthreshold stimulus to the efferent axon, this responds with a local potential of variable height and either aperiodic or with damped oscillations. When the preephaptic action currents reach a threshold value a spike is discharged along the postephaptic axon, 2 to 5 msec. after the afferent spike reaches the contact zone. This interval is occupied by the rise of the local active prepotential to a threshold value. Depending on the state of the effector axon, an after-discharge may or may not follow this first spike.

Additional phenomena appear when the efferent axon displays an initial oscillatory activity of its own. In this case, a subthreshold afferent spike brings about incrementing of the spontaneous oscillations of the efferent axon; and often, after a few such increasing oscillations, the discharge of a spike. As the stimulating value of the afferent impulse increases to threshold, the delay at the ephapse shortens and finally becomes less in the cases considered in the preceding paragraph. If the autonomous activity of the efferent axon includes a periodic discharge of spikes, the introduction of an extra-response, by means of an afferent spike, causes a break in this rhythm. An early extra-response is followed by a prolonged cycle, as if there were a compensatory pause.

The ephaptic phenomena have been discussed in relation to synaptic phenomena and to other interaction phenomena between central cells, particularly those involved in synchronization.

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LOCALIZATION OF ENZYMES IN NERVES
I. SUCCINIC DEHYDROGENASE AND VITAMIN B_1

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INTRODUCTION

RECENT investigations have shown that the enzyme choline esterase is found in high concentrations in the sheath of the giant axon of the squid, only a negligible amount being present in the bulk of the axoplasm (2, 14). This finding is of particular interest in view of the parallelism found between electrical activity and concentrations of the enzyme in electric organs, and suggests that there is a correlation between acetylcholine metabolism and electrical activity in general (15).

In view of the high concentration of choline esterase at the neuronal surface it becomes important to determine the distribution of other enzyme systems known to be of consequence in the general metabolism of the cell as well as in the specific processes probably involved in excitation and transmission. The present paper deals with the distribution in squid nerves of succinic dehydrogenase and oxidase systems and with the vitamin B_1 in its phosphorylated form, diphosphothiamin.

METHODS

a. *Succinic dehydrogenase.* The method of Quastel and Wheatley (1938) has been used. The test depends on the reduction of ferricyanide by the hydrogen of succinic acid. For one reduced molecule of ferricyanide one molecule of acid is formed, and in bicarbonate solution this gives rise to one molecule of CO_2, which is estimated manometrically. According to the equations for the reactions involved:

$$H + Fe(CN)_6''' \rightarrow H^+ + Fe(CN)_3''''$$
$$H^+ + HCO' \rightarrow H_2CO_3 \rightarrow CO_2 + H_2O$$

each molecule of succinic acid will liberate two molecules CO_2.

The amounts of tissue available were small and, at room temperature, lower rate of reaction could be expected than in the work of Quastel and Wheatley. In order to increase the sensitivity of the method, small conical vessels with side bulb were used with a total volume of about 7 cc. The KCO_2 of these vessels at room temperature was 0.6–0.7, if about 1 cc. fluid was present. In the main compartment of the vessel there was placed 1 cc. of Ringer-$NaHCO_3$ (0.025 M), and in the side bulb 0.1 cc. of the ferricyanide solution (5 cc. of a 10 per cent sodium ferricyanide solution with 1 cc. 0.16 M $NaHCO_3$). The gas mixture used was 95 per cent N_2 + 5 per cent CO_2. All experiments were carried out at 22–23°C.

Sheath and axoplasm samples of giant fibers of the squid were always prepared from two pairs of fibers, one used as control without succinate the other with succinate. The separation of sheath and axoplasm was carried out as previously described (18). Samples were weighed on a microbalance and then finally minced in the manometer vessels. Only the difference of the CO_2 output between experimental and control value was considered as a measure of the activity of succinic dehydrogenase.

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b. *Succinic oxidizing system.* Oxygen uptake was determined with the same vessels as used for succinic dehydrogenase and the material was prepared in the same way. As buffer, 1.5 cc. of a 0.1 M phosphate solution were used with a pH of 7.3. The succinate concentration was always 0.15 M. 1.5 mg. of cytochrome dissolved in 0.1 cc. were added to each vessel in order that an insufficient concentration of cytochrome should not be the limiting factor. The cytochrome was prepared as described by Keilin and Hartree (6). The determinations were carried out in air. The difference of the O_2 uptake with and without succinate was considered as a measure of the activity of succinic oxidizing system.

c. *Cocarboxylase* (*diphosphothiamin*). The method of Lohmann and Schuster (9) was used. This method is based on the principle that the carboxylase in washed yeast extract will decarboxylate pyruvic acid only in presence of diphosphothiamin. If to a washed yeast preparation which has practically no decarboxylating action, a tissue extract is added and the CO_2 output compared with the CO_2 output in the presence of known concentrations of diphosphothiamin, the amount of this substance in the extract can be calculated by interpolation.

The method has been used with slight modifications of the original. 1 g. well dried and ground yeast was weighed into a centrifuge tube and about 50 cc. H_2O were added. The tube was shaken vigorously at intervals for 20 min., after which the suspension was poured into another centrifuge tube and centrifuged for 5 min. and the supernatant fluid discarded. The yeast was then washed twice with about 50 cc. of 0.1 M dibasic phosphate at 30°C., and then once with distilled water. Each of the three washings was carried out within 4 min., centrifuging included. 8 cc. of phosphate buffer of a pH of 6.2 were then added and 0.8 cc. of this suspension put into each vessel together with 1 cc. of extract or known diphosphothiamin solution.

In the side bulb were placed 0.2 cc. of a solution, containing 0.2 mg. Mg, 10μg. thiamin and 5.0 mg. sodium pyruvate. This latter was freshly prepared for each experiment: about 50 mg. were weighed and a corresponding amount of the stock solution of Mg and thiamin were added. After the temperature equilibrium was reached the solution of the side bulb was tipped into the main vessel. The reading after the first 5 min. was discarded.

Results

A. *Succinic dehydrogenase*

The ultimate source of energy used in living cells is oxidation and most cellular functions consume energy. Conduction of nerve impulses requires energy: it is connected with heat production and extra oxygen uptake (3, 4, 5). But two restrictions have to be made: (i) Oxidation is a slow process. It is known, from investigations on muscle, that oxidation is not connected directly with contraction but occurs during recovery (12). It is difficult to believe that the extra oxygen uptake in nerves is directly connected with conduction, an event far more rapid than muscular contraction. (ii) The extra oxygen uptake during nerve activity is small compared with the oxygen uptake of the resting nerve, in contrast with the important extra oxygen uptake following muscular activity. Whatever the role of oxidation in conduction may be over all oxygen consumption is certainly connected with many other activities of the nerve cell. It therefore can be expected that the localization of respiratory enzyme systems differs essentially from that of choline esterase.

It is widely believed that succinic dehydrogenase is an essential intermediate in respiration. The work of Szent-Györgyi (19) and his associates suggests that the succinic-fumaric acid system is directly linked to the cytochrome-cytochrome oxidase system. The activity of succinic dehydrogenase has been determined in the head ganglion of squids, where cell bodies

Table 1. Succinic dehydrogenase in the head ganglion at different concentrations of succinate, varying from 0.075 to 0.30 M. All figures are calculated for 100 mg. fresh tissue.

	Control 30.5 mg.	0.075 M 25.0 mg.		0.15 M 26.0 mg.		0.30 M 26.0 mg.	
min.	cmm. CO_2	cmm. CO_2 (corr.)	µg. succinic acid	cmm. CO_2 (corr.)	µg. succinic acid	cmm. CO_2 (corr.)	µg. succinic acid
70	60.0	206	*542*	228	*600*	199	*524*
185	95.0	431	*1135*	472	*1240*	415	*1090*

and synapses are located, in whole giant axon and in sheath and axoplasm separately. The enzyme activity was also determined in the whole trunk containing, besides the giant axon, many fibers of small diameter and therefore relatively larger surface.

Table 1 gives the values obtained with the head ganglion. The control values indicate the CO_2 liberation per 100 mg. of fresh tissue without

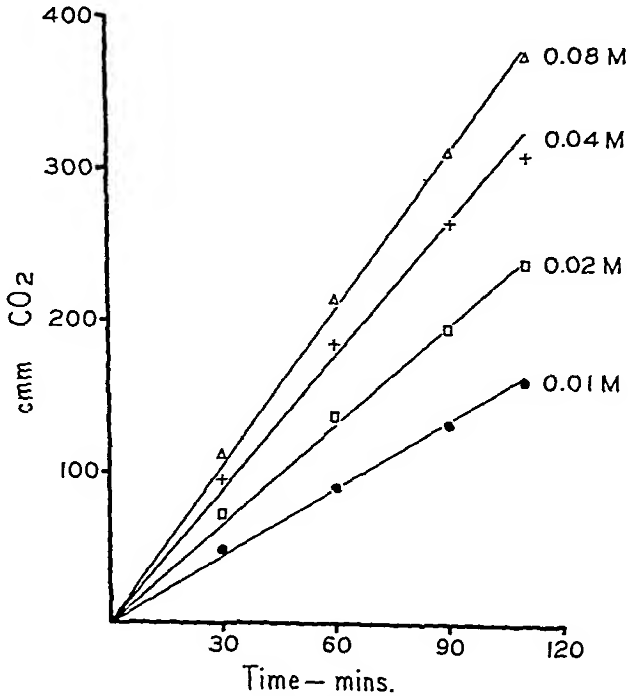

FIG. 1. Activity of succinic dehydrogenase in the head ganglion of the squid at different concentrations of succinate. Abscissae: cmm. CO_2 liberated by 100 mg. fresh tissue (corrected). Ordinates: time of observation in min.

succinate. From the experiments with succinate are given the figures of CO_2 production calculated for 100 mg. of fresh tissue after subtraction of the

Table 2. Succinic dehydrogenase in the whole giant axon of squids.

Exp. Nr		Control 22.0 mg.	Experiment 23.0 mg.		
		cmm. CO_2	cmm. CO_2	μg. succinic acid	
	min.	per 100 mg.	per 100 mg. (corr.)	absol.	per 100 mg.
1	80	7.4	21.3	*12.9*	56.0
	160	10.3	44.9	*27.1*	118.0
	255	13.3	65.5	*39.6*	172.0
	320	14.8	78.2	*47.3*	206.0
		Control 30.0 mg.		Experiment 28.0 mg.	
2	80	6.6	19.1	*14.1*	50.2
	160	9.8	40.9	*30.1*	107.5
	230	9.8	54.8	*40.5*	144.5

control and the corresponding amount in μg. succinic acid dehydrogenated. At a concentration of 0.01 M of succinate used by Quastel and Wheatley the activity rate in these experiments is far from being optimal (Fig. 1). The values in Table 1 indicate that 0.15 M is about optimal. This concentration was used in experiments with the giant fiber.

The activity in the giant fiber is considerably smaller, less than 10 per cent of that in the head ganglion. In the whole giant axon about 50 μg. of succinic acid were metabolized in 80 min. at 0.15 M concentration per 100 mg. fresh tissue as compared with 670μg. in the head ganglion during the same time, at the same substrate concentration and the same temperature (Table 2). In the whole trunk the amount was even smaller: only 37 μg. per 100 mg. fresh tissue (Table 3).

If the activity of succinic dehydrogenase is determined in sheath and axoplasm separately, the most important fact is that in absolute amounts about 90 per cent of the enzyme is located in the axoplasm (Fig. 2). The

Table 3. Succinic dehydrogenase in the nerve trunk containing the giant axon.

	Control 94.0 mg.	Experiment 83.0 mg.		
	cmm. CO_2	cmm. CO_2	μg. succinic acid	
	per 100 mg.	per 100 mg. (corr.)	absol.	per 100 mg.
80 min.	7.0	14.0	*31*	37
160 min.	10.7	25.0	*55*	66
230 min.	15.2	30.3	*65*	80

values obtained with sheath are in fact so small that they are within the limits of sensitivity of the method. In Table 4 the observed CO_2 values are given and not those calculated for 100 mg. of tissue as in the previous tables, because in this case the absolute amounts are essential. The figures show that

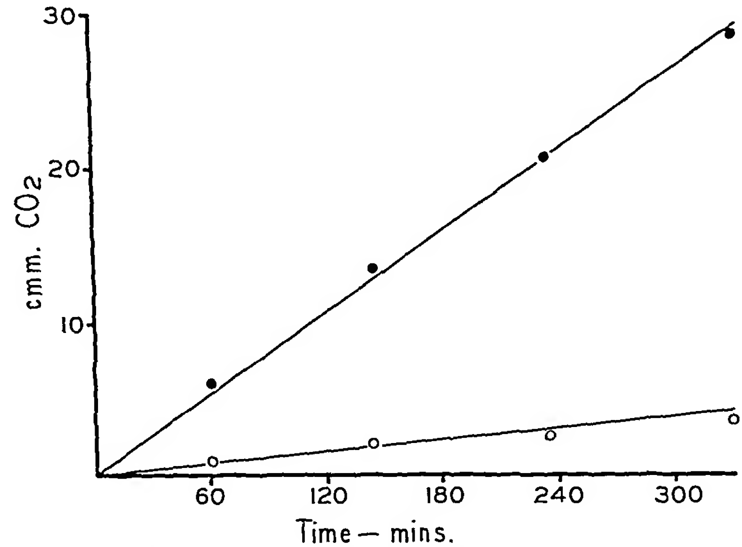

FIG. 2. Succinic dehydrogenase in sheath and axoplasm of the giant fiber of the squid. Abscissae: cmm. CO_2 liberated. Absolute amounts (corrected). Ordinates: time of observation in min. ●——● axoplasm, ○——○ sheath.

Table 4. Succinic dehydrogenase in sheath and axoplasm of the giant fiber of squids.

		Sheath				Axoplasm			
		Control 5.6 mg.	Experiment 5.2 mg.			Control 17.6 mg.	Experiment 20.9 mg.		
	min.	cmm. CO_2 observ.	cmm. CO_2 observ.	μg. succinic acid absol.	μg. succinic acid per 100 mg.	cmm. CO_2 observ.	cmm. CO_2 observ.	μg. succinic acid absol.	μg. succinic acid per 100 mg.
I	80 145 235	0.65 1.63 1.95	1.50 2.41 2.71	*2.3* *2.3* *2.4*	44 44 46	3.8 5.5 7.6	9.9 15.8 23.6	*14.1* *24.3* *38.6*	67.5 116 185
		Control 6.7 mg.	Experiment 5.5 mg.			Control 27.5 mg.	Experiment 24.9 mg.		
II	60 145 235 330	0.65 1.63 1.95 1.95	1.50 3.31 4.21 5.41	*2.6* *5.2* *6.9* *9.3*	47 95 126 169	2.7 3.8 4.5 5.2	8.5 17.0 24.5 32.8	*17.9* *35.5* *53.8* *73.7*	72 142.5 216 296

the enzyme activity in the sheath is hardly measurable. If we accept them as an approximate indication of the enzyme activity in the sheath the concentration (amount metabolized per 100 mg. wet weight) is about half of that in the axoplasm. A considerable part of the sheath is connective tissue, which has no enzymatic activity. Some axoplasm may remain attached to the sheath although most of it has been squeezed out. It is still possible that the actual concentration of the enzyme is somewhat higher in a very thin layer at or near the nerve surface. These experiments do not offer any evidence for such a possibility. The same considerations can be applied to the low values obtained with the whole trunk which contains on the one hand small fibers with a relatively large surface and on the other hand more connective tissue than the giant axon. It is, however, certain that the distribution of succinic dehydrogenase is in striking contrast to that of choline esterase.

Table 5. Succinic oxidizing system in the head ganglion of squids. All figures are given for 100 mg. of fresh tissue. C = Control, E = Experiment, S.A. = succinic acid.

Exp. No.:	I			II			III			IV			V		
	C 36.5 mg.	E 43.5 mg.		C 42.5 mg.	E 42.5 mg.		C 40.0 mg.	E 38.5 mg.		C 34.5 mg.	E 38.0 mg.		C 33.5 mg.	E 35.0 mg.	
min.	O_2 cmm.	O_2 cmm. (corr.)	S.A. mg.	O_2 cmm.	O_2 cmm. (corr.)	S.A. mg.	O_2 cmm.	O_2 cmm. (corr.)	S.A. mg.	O_2 cmm.	O_2 cmm. (corr.)	S.A. mg.	O_2 cmm.	O_2 cmm. (corr.)	S.A. mg.
60	14.0	116	*0.61*	18.5	94.5	*0.50*	16.2	115	*0.60*	17.8	105	*0.55*	11.6	110	*0.58*
120	19.0	211	*1.11*	26.0	183	*0.96*	24.3	211	*1.11*	26.8	203	*1.07*	17.9	225	*1.18*
180	—	—	—	—	—	—	30.0	297	*1.56*	34.6	288	*1.52*	22.5	322	*1.69*
240	26.6	350	*1.84*	38.4	326	*1.72*									

B. *Succinic oxidizing system*

The final oxidation of succinate requires, besides succinic dehydrogenase and other intermediate links, the cytochrome-cytochrome oxidase system. Succinic dehydrogenase is the limiting factor of the rate of the reaction, but as in most cells it is present in rather large amounts, oxidation of succinic acid may give an approximate indication for the distribution of cytochrome oxidase. It shows in any case whether succinic acid can be oxidized at the same rate as it is dehydrogenated.

In five experiments oxidation of succinic acid has been determined in the head ganglion (Table 5). Cytochrome c was added in excess thus ensuring that an insufficient concentration of this substance would not be a limiting factor. About 500–600 μg. succinic acid were oxidized in 60 min. by 100 mg. fresh tissue. This amount corresponds to the amount dehydrogenated by succinic dehydrogenase in anaerobiosis. It is calculated on the assumption that one molecule oxygen oxidizes one molecule succinic acid. This means that the reaction stops with the formation of hydrogen peroxide, no catalase

being present to split the H_2O_2. No experiments have been made to test whether catalase is present or not. But as it is hardly possible that two molecules of succinic acid are oxidized for 1 molecule dehydrogenated, the calculation based on the assumption of H_2O_2 formation appears justified.

The activity of the succinic oxidizing system in sheath and axoplasm of the giant fiber is the same as that found for succinic dehydrogenase. The calculation is again based, as for the head ganglion, on the assumption of H_2O_2 formation. Only one experiment has been made due to shortage of material at the advanced season when the experiments were made. But it appears significant as all experiments with the head ganglion preparations show that the amounts of succinate metabolized are the same in aerobic and anaerobic conditions and there is no reason for assuming that the ratio of the two enzymes in the fiber is different. Figure 3 demonstrates that the oxidation of succinic acid in the giant axon parallels the rate of its dehydrogenation shown in Fig. 2.

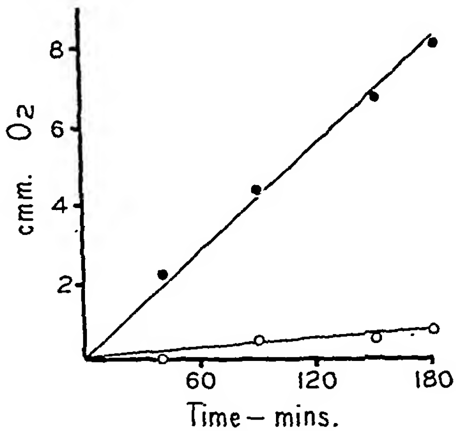

FIG. 3. Succinic oxidizing system in sheath and axoplasm of the giant fiber of the squid. Abscissae: absolute amounts of O_2 taken up (corrected). Ordinates: time of observation in min. ●——● axoplasm, ○——○ sheath.

C. *Vitamin B_1 (Diphosphothiamin)*

Oxidation of pyruvic acid goes through formation of acetic acid (1, 8, 13, 16). Acetylation of various substances occurs easily subsequent to pyruvic acid oxidation. It is therefore probable that the acetic acid of acetylcholine is derived from pyruvic acid. Evidence for this assumption was brought out in experiments of Quastel and his colleagues who found that formation of acetylcholine in brain slices occurs only in oxygen in presence

Table 6. Succinic oxidizing system in sheath and axoplasm of the giant fiber of squids.

	Sheath				Axoplasm			
	Control 4.1 mg.	Experiment 3.7 mg.			Control 22.1 mg.	Experiment 22.4 mg.		
min.	O_2 cmm. observ.	O_2 cmm. observ.	μg. succinic acid absol.	μg. succinic acid per 100 mg.	O_2 cmm. observ.	O_2 cmm. observ.	μg. succinic acid absol.	μg. succinic acid per 100 mg.
40	1.05	0.97	*0.12*	3.16	1.13	3.43	*12.0*	54
90	1.05	1.45	*2.64*	71.8	1.40	5.81	*23.1*	103
150	1.84	2.17	*2.69*	72.8	1.97	8.71	*35.4*	158
180	1.84	2.41	*3.94*	107.0	2.25	10.30	*42.4*	189

Table 7. Cocarboxylase in head ganglion and giant axon of squids.

Exp. No.	tissue	mg. fresh tissue	μg. cocarboxylase absol.	μg. cocarboxylase per 100 mg.
1	head ganglion	159.0	2.10	*1.32*
2	head ganglion	176.0	2.15	*1.22*
3	2 head ganglia	328.0	4.14	*1.26*
4	whole giant-axon	52.7	0.055	*0.105*

of either glucose or pyruvate. Since it is well known from the work of Peters and his associates that the oxidation of pyruvic acid requires vitamin B_1, Mann and Quastel (11) investigated whether the formation of acetycholine in brain slices, kept in pyruvate-Locke-bicarbonate media, is accelerated in presence of vitamin B_1. In brains of normal pigeons vitamin B_1 had no effect, but in vitamin deficient pigeons addition of vitamin B_1 increased definitely the rate of acetylcholine formation, although only in presence of a high potassium concentration (0.03 M).

If acetylcholine formation requires vitamin B_1 it appeared possible that, in view of the high acetylcholine metabolism at or near the surface of nerve fibers, vitamin B_1 was concentrated there as well. However, it would not be expected that vitamin B_1 is localized near the surface as exclusively as choline esterase because pyruvic acid oxidation is an important intermediate step for many reactions other than acetylcholine formation.

The active form of vitamin B_1 in cells is the diphosphothiamin (cocarboxylase) and most of the vitamin B_1 in tissues seems to occur in this form (8). The cocarboxylase has been determined in the head ganglion and in sheath and axoplasm of the giant fiber in order to compare its distribution with that of choline esterase. Extracts were prepared of sheath and axoplasm by grinding in quartz tubes with quartz powder and water. The tubes were

Table 8. Cocarboxylase in sheath and axoplasm of the giant fiber of squids.

	Control	Sheath 14.0 mg.			Axoplasm 87.0 mg.			Standard 0.05 μg. coc.	
	CO_2 c.mm.	CO_2 c.mm.		μg. coc.	CO_2 c.mm.		μg. coc.	CO_2 c.mm.	
min.	observ.	observ.	per 100 mg. (corr.)	absol.	observ.	per 100 mg. (corr.)	absol.	observ.	corr.
10	1.00	2.36	9.7	*0.0356*	4.09	3.6	*0.0807*	2.91	1.91
15	1.66	3.84	15.6	*0.0342*	6.81	5.9	*0.0807*	4.85	3.19
20	2.66	5.32	19.0	*0.0303*	9.52	7.9	*0.0781*	7.05	4.39
25	3.65	6.80	22.5	*0.0310*	11.6	9.2	*0.0784*	8.73	5.08
30	3.98	8.00	28.7	*0.0299*	14.0	11.5	*0.0746*	10.70	6.72

average value of 5 readings: *0.0322* *0.0785*
per 100 mg: *0.230* *0.090*

then immersed in boiling water for 3 min. following which they were stored at low temperature until used.

1.2–1.3 μg. cocarboxylase per 100 mg. fresh tissue have been found in the head ganglion (Table 7). This is a value several times as high as that of Ochoa and Peters for pigeon's and rat's brains (2.5–4.0 μg. per g. fresh tissue). In the the whole giant axon the value found was rather low, less than 10 per cent of that in the head ganglion: 0.105 μg. per 100 mg. fresh tissue.

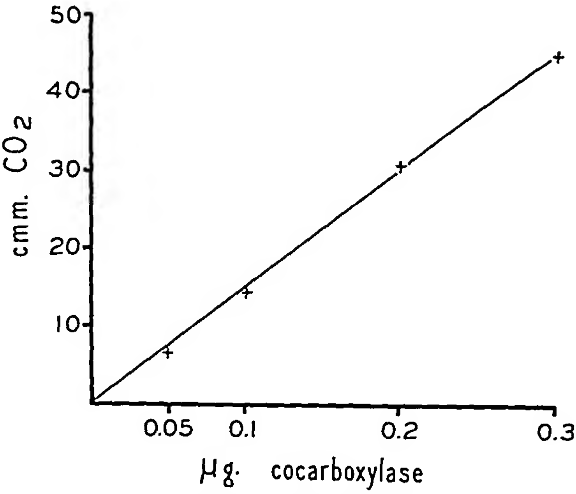

FIG. 4. CO_2 developed in 30 min. at 4 different concentrations of cocarboxylase.

In view of the low concentration of cocarboxylase in the fiber and the small amounts of tissue available only small quantities of cocarboxylase could be expected in the extracts prepared. The method had therefore to be adapted to such small quantities. This did not represent any particular difficulty (see details under methods). Table 8 gives one complete experiment. Rates calculated from readings at different times show a satisfactory agreement. If four different concentrations of the standard solution (0.05, 0.1, 0.2, and 0.3 μg.) are plotted at a given time against the CO_2 liberated, a straight line is obtained (Fig. 4). In order to test the sensitivity of the method one experiment has been carried out with amounts of 0.05, 0.07 and 0.1 μg. cocarboxylase. Figure 5 shows that even with such low quantities the difference of the various concentrations is sufficiently marked and that if the amounts of cocarboxylase are plotted against the amount of CO_2 liberated at a given time a straight line is obtained.

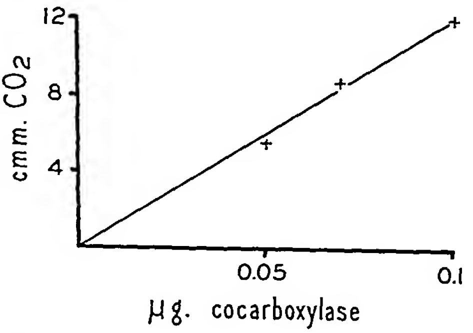

FIG. 5. CO_2 developed in 30 min. with small amounts of cocarboxylase.

The figures show that the concentration of cocarboxylase is higher in the sheath than in the axoplasm. The *absolute* amount per nerve is somewhat higher in the axoplasm because of the greater mass of material. The difference of concentration between sheath and axoplasm is shown in Fig. 6. In Table 9 the results of 4 experiments are given. The best time of observation is about 25 min. However, the first experiment was continued only 15 min.

after substrate and enzyme were mixed and in the second experiment no reading was made at 25 min. All four experiments show that the concentration of cocarboxylase in the sheath is about 2–3 times as high as that in the axoplasm. In absolute amounts the figures of experiment no. 3 are lower than those of the other experiments. This is probably due to the fact that these extracts were kept for nearly a week in order to collect a maximal amount of material. It is probable that during such a long time a certain dephosphorylation occurred and that therefore the extracts yielded low values.

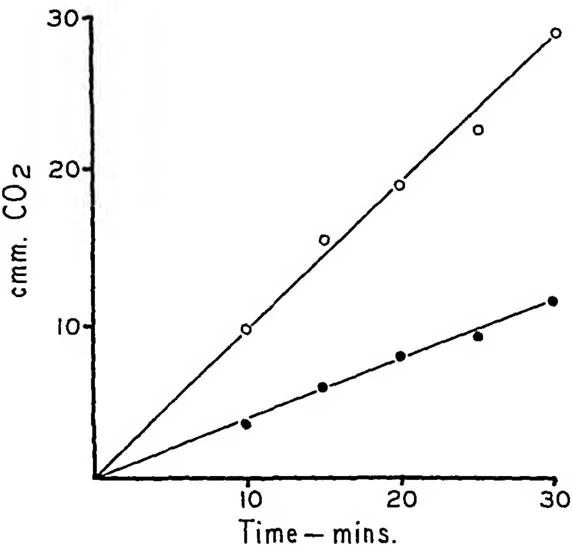

FIG. 6. Concentration of diphosphothiamin (cocarboxylase) in sheath and axoplasm of the giant fiber of the squid. Abscissase: cmm. CO_2 liberated by extracts calculated for 100 mg. of fresh tissue. Ordinates: time of observation in min. ●——● axoplasm, ○——○ sheath.

If the *concentration* in the sheath is 2–3 times higher than that in the axoplasm the real difference must be still greater; at least 50 per cent of the sheath used is connective tissue, probably more, and a part of the axoplasm may adhere to the sheath thereby decreasing the apparent concentration. A cautious estimate would be that

Table 9. Cocarboxylase in sheath and axoplasm of the giant fiber of squids.

Exper. No.	part of fiber	mg. fresh tissue	min. of observ.	μg. cocarbox. absol.	μg. cocarbox. per 100 mg.
1.	Sheath	15.2	15	0.0498	*0.327*
	Axoplasm	87.0		0.0476	*0.055*
2.	Sheath	13.1	30	0.0354	*0.270*
	Axoplasm	72.5		0.0745	*0.103*
3.	Sheath	22.5	25	0.0313	*0.139*
	Axoplasm	110.0		0.0768	*0.070*
4.	Sheath	14.0	25	0.0310	*0.221*
	Axoplasm	87.0		0.0784	*0.090*

the concentration of cocarboxylase at or near the surface is at least 5–10 times as high as in the axoplasm.

Discussion

The localization of choline esterase at or near the neuronal surface is an essential support for the conception that acetylcholine metabolism is intrinsically connected with the electrical changes occurring during nerve activity. If the localization of this enzyme is to be accepted as significant, the distribution of other enzymes less closely connected with activity should differ from that of choline esterase. The distribution of the enzymes studied so far and described in this paper supports this assumption: the greater part of succinic dehydrogenase and oxidase, widely considered as important links in the respiratory enzyme system, is located in the axoplasm. Although it is not certain whether the whole respiration passes through the succinic-fumaric acid system as originally suggested by Szent-Györgyi, it may be taken as an approximate indication for the distribution of respiratory enzymes. This agrees well with the assumption that oxidation is most probably not as closely and specifically connected with nerve conduction and transmission as acetylcholine metabolism and that oxidation, whatever its significance for conduction may be, is important for most cell activities. It is, however, desirable to test other important links, especially cytochrome oxidase, before drawing a final conclusion about the distribution of the respiratory enzymes. Lack of material, with the method used, made it impossible to study other respiratory enzymes during this season. The studies will be continued with a more adequate micro-respiration technique. In any case the contrast to the distribution of choline esterase is very marked.

The distribution of vitamin B_1 as diphosphothiamin, its active form, differs both from that of choline esterase and from that of succinic dehydrogenase. Its concentration is definitely higher in the sheath although the absolute amounts are somewhat higher in the axoplasm which contributes the greatest bulk of the whole nerve. It is suggested that this concentration is connected with the fact that oxidation of pyruvic acid is necessary for acetylation of choline, a process requiring vitamin B_1. This assumption may explain the sensitivity of the nervous system to vitamin B_1 deficiency: For if acetylcholine is essential for conduction and transmission of nerve impulses a decrease in the rate of its formation may lead to the polyneuritis observed in this deficiency.

Whatever the interpretation of this distribution may be, the fact itself, that the localization of the three enzymes studied differs so greatly, appears significant and emphasizes the specificity of the localization of choline esterase.

Summary

Studies have been initiated to determine the distribution in the nerve cell of different enzymes possibly important for nerve activity and to compare it with the distribution of choline esterase. The enzyme activities have been determined in sheath and axoplasm of the giant fiber of squids and in the

head ganglion. So far succinic dehydrogenase, the succinic oxidizing system and vitamin B_1 as diphosphothiamin have been determined.

(1) About 90 per cent of succinic dehydrogenase of the giant axon are located in the axoplasm. The experiments bring no evidence for the assumption that the enzyme is concentrated at or near the surface. The distribution is in striking contrast to that of choline esterase. In the head ganglion the concentration of succinic dehydrogenase is about 10 times as high as that in the fiber. The oxidation of succinic acid occurs at the same rate as its dehydrogenation.

(2) Diphosphothiamin is concentrated several times more in the sheath than in the axoplasm. This fact supports the assumption that vitamin B_1 is required for the formation of acetic acid for the acetylcholine from pyruvic acid. The distribution differs from that of choline esterase as well as from that of succinic dehydrogenase.

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CEREBELLAR ACTION POTENTIALS IN RESPONSE TO STIMULATION OF THE CEREBRAL CORTEX IN MONKEYS AND CATS*

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INTRODUCTION

ELECTRICAL stimulation in the region of the pons results in action potentials in cat's cerebellum, in lobulus ansiformis, paraflocculus, lobulus paramedianus, folium and tuber vermis, culmen, lobulus simplex and pyramis (Dow, 1939). Curtis (1940) stimulated the cerebral cortex in cats and obtained action potentials in the cerebellar cortex in the same lobules, except that none was found in paraflocculus nor the lobules of vermis posterior to lobulus simplex. The corticopontocerebellar system is best studied in primates, for in these animals this afferent cerebellar system has its greatest development. The present report deals with the responses in various lobes of the cerebellum following electrical stimulation of the major subdivisions of the cerebral cortex in monkeys and cats.

MATERIAL AND METHODS

Observations were made on 8 monkeys and 11 cats. Most of the animals were anesthetized with nembutal (1 cc. 6.5 per cent solution per 2.27 kg.) given intra-peritoneally. In a few ether anesthesia was used throughout the experiment. In the experiments in which the anterior lobe of the cerebellum was explored with the lead electrode the occipital lobe of one cerebral hemisphere was ablated and the tentorium cerebelli laid back to expose the entire culmen on that side. Stimulation was produced by a single condensor discharge of relatively short duration (0.3 msec.) delivered through an audio-transformer to reduce the shock artifact. The stimulating electrode consisted of a glass bead in which two wires were buried. The glass was then ground until the ends of the wires were flush with the flat surface of the electrode. The exposed surfaces of the wires were about 1.5 mm. apart. This was lightly applied to the surface of the brain by means of a mechanical manipulator. The strength of the shock used to explore the cortex with these electrodes was 140 V. peak voltage. This was approximately 20 times the threshold in a zone of lowest threshold. This strong shock, when delivered through the glass bead electrode, never produced visible spread to nearby muscles, and the response could be abolished by the local application of cocaine solution. When silver wire electrodes 2 to 3 mm. apart were applied to the cortex, evidences of spread of current to adjacent tissues were observed when the voltage was above 50 V. The essentially local effect of the strong shock delivered through the glassed electrodes was further shown by the fact that a movement of only 2 or 3 mm. would frequently radically change the cerebellar response and in certain areas cause it to disappear completely.

Lead electrodes consisted of a pointed wick of cotton wool whose contact surface was rarely greater than 2 mm. in diameter and usually about 1 mm. An indifferent electrode was placed on the skull or on the skin of the neck. In certain experiments two leads were taken from the same cerebellar lobule with two wicks of cotton or with two silver wires a few mm. apart. This was in order to be doubly sure that the potentials were the result of nervous activity at the lead site. In some of the experiments a double channel cathode ray

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oscillograph was used so that the separate responses at two points could be observed from an identical stimulus.

Results

Cat. Cerebellar action potentials were observed following stimulation of the opposite cerebral hemisphere in every experiment. The lobes of the cerebellum in which there were responses were culmen, lobulus simplex, lobulus ansiformis, folium and tuber vermis, pyramis, lobulus paramedianus and paraflocculus, This represents all the folia which may be exposed on the surface of the cerebellum of the cat except the uvula. Action potentials were never observed from leads on the uvula in the cat. Figure 1C shows the potentials obtained from some of these lobes. These records were taken with both leads on the same lobule of the cerebellum only a few millimeters apart. This accounts for the complex wave form in some of the leads. Although the crest of the major deflection occurred about 25 msec. after the shock artifact, it is obvious that there is local activity appearing much earlier, in fact as early as 3 msec. It was regularly observed that responses resulting from stimulation of just above threshold strength consisted only of the wave at the 25 msec. latency. As the strength of the shock was increased to several times threshold, the earlier response would then appear. Although a much later response, occurring after a latency of 200 msec. (Curtis, 1940), was frequently watched

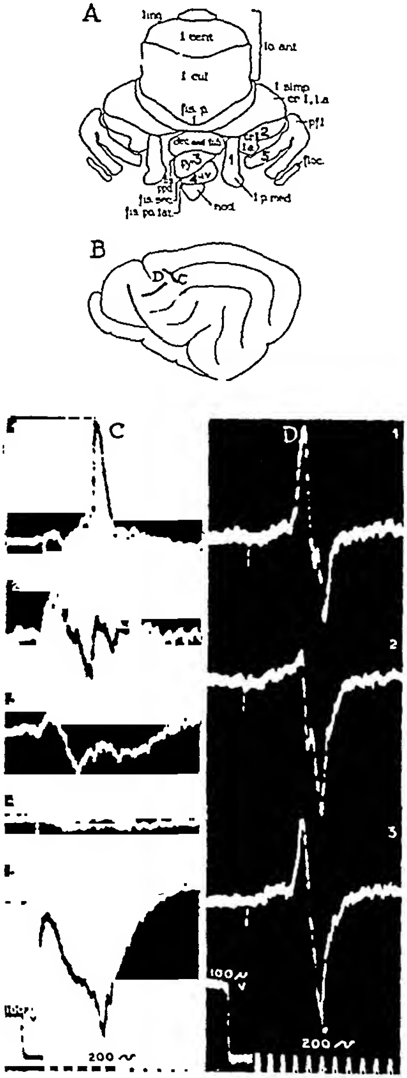


Fig. 1. A. Schema of the cat's cerebellum. Numbers 1–5 refer to corresponding numbers in C and indicate the lead points chosen for the records. Stimulus identical in strength and location. B. Diagram of the left cerebral hemisphere in the cat. C indicates stimulus point for records C 1–5. D indicates the point at which needle electrodes were thrust 4 mm. into subcortical white matter. At this depth, stimulation with low voltage A.C. current gave an isolated movement of thigh muscles. Single shock stimulation here produced action potentials shown in D. C. Bipolar surface lead on (1) paramedianus; (2) Crus II lobulus ansiformis; (3) pyramis; (4) uvula; (5) paraflocculus. D. Three responses on lobulus paramedianus from identical stimuli applied to the same subcortical point at about 4 sec. intervals. Note differences in form of each response. Bipolar recording.

for on slower sweep speeds, it appeared only rarely, and then was so inconsistent in appearance as to make its interpretation difficult. No statement can be made from this study concerning the presence or absence of potentials from the folia of the base of the vermis and flocculus, but following stimulation of the pons in the cat there were none in these lobules (Dow, 1939).

The sigmoid, coronal, lateral, suprasylvian, ectosylvian and sylvian gyri of the cerebral cortex were stimulated. Responses appeared in widely scattered parts of the cerebellar cortex from stimulation of a single point on the cerebral cortex. The most marked and most consistent responses and those at lowest threshold resulted from stimulation in the sigmoid, coronal, anterior ectosylvian and anterior part of the lateral, suprasylvian and middle ectosylvian gyri. A single response found only in the pyramis was obtained from the posterior ectosylvian gyrus and a point in the posterior suprasylvian gyrus. In another isolated experiment there was a response in the lobulus paramedianus from a stimulus in the posterior part of the middle ectosylvian gyrus. There were no responses on any part of the cerebellum in any experiment from stimulation of the sylvian, the posterior lateral and posterior part of the middle lateral gyrus. In a few experiments ether anesthesia was substituted for nembutal, and the depth of the anesthesia was varied from a light stage to respiratory arrest without changing the gyri giving responses to the lobulus paramedianus. This, however, does not prove that connections might not be established by less direct route from other areas which in the conditions of our experiments failed to give responses. The connections demonstrated here may represent only the most direct connections and not the total possibilities.

The responses were the largest and most readily obtained in the lobulus ansiformis, lobulus paramedianus and paraflocculus. Culmen, folium and tuber vermis and pyramis were somewhat less apt to show action potentials, but even here the responses occurred from widely scattered parts of the cerebral cortex. A comparison was made of the responses in the various cerebellar lobules when points within topographical subdivisions of the cat's cortex were stimulated. Face, arm and leg areas either were determined by stimulation with low voltage alternating current, or points were selected on the basis of Adrian's (1940, 1941) map of the somatic sensory areas. The cerebellar lobes which showed action potentials were the same regardless of which topographical area was stimulated, and no difference in the threshold or in the amplitude of the responses could be detected.

Although the sign of the potential when a "monopolar" lead was placed on the surface of the cerebellum was usually surface positive, there were frequently important surface negative elements in the potential. It was not possible to predict what the sign or form of the potential might be. Sometimes a shift of the stimulation electrode to a new point might reverse completely the sign of all components of the potential recorded from an identical cerebellar lead point without disturbing the time sequence of any part of

the potential as measured from the shock artifact. The form of the potential frequently was different for different stimulation points with identical lead points and even from a series of stimuli applied to the same point in rapid succession. These differences were naturally accentuated when leading with bipolar electrodes, but might be present also with single active electrodes. A stimulation by needle electrodes of the subcortical white matter (Fig. 1D) elicited this also.

The response can be inhibited for relatively long periods by a previous shock at the same cerebrocortical point. This inhibitory effect may be present

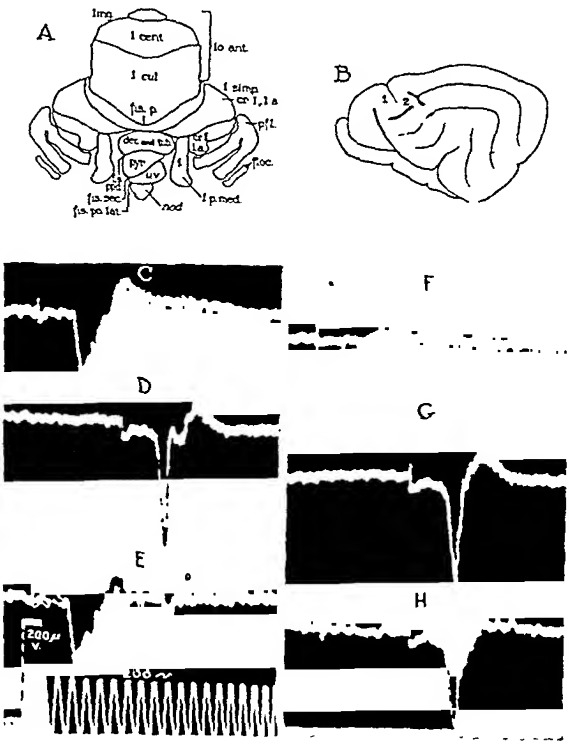


FIG. 2. A. Schema of the cat's cerebellum. Point 1 shows lead on right lobulus paramedianus used foı records C–H. B. Left cerebral hemisphere of the cat. (1) Stimulus point for conditioning shock, hind limb area 4. (2) Stimulus for testing shock, forelimb area 4. C. Conditioning response alone. Stimulus strength 2× threshold. D. Testing shock alone. Stimulus strength 2× threshold. E. Both. Note almost complete elimination of testing response at 32 msec. interval. F. Conditioning alone after local application of 5 per cent cocaine at point 1 for 5 minutes. Stimulus strength as in C. Note elimination of response. G. Test alone. Stimulus strength as in D. Comparison with D shows it to be unaffected by cocaine. H. Both. Note almost complete absence of effect on testing shock.

In these and all subsequent records a deflection down is a surface positive potential.

for as long as a second. Conversely the size of the response frequently increased from shock to shock, even though successive shock were delivered about 4 sec. apart. A detailed investigation of factors for facilitation and inhibition has not been made, but it is obvious that the corticopontocerebellar system is subject to facilitation and inhibition as are other types of cortical activity. Stimuli applied to widely separated cortical points in separate topographical subdivisions can completely abolish each other for many milleseconds (Fig. 2C–E). One cannot be certain from the data at hand that this is not due to intracortical connections. By the use of cocaine at the site of stimulation it was shown that current spread was not responsible for the conditioning effect (Fig. 2F–H).

Monkey. The cerebellar divisions from which responses were obtained in *Macaca mulatta* correspond closely to those in the cat. The responses differed only in that those on the culmen were on the whole larger than in the cat, and in one experiment significant potentials were present in the uvula. As in the cat, there were responses also in lobulus simplex, lobulus ansiformis, lobulus paramedianus, paraflocculus, folium and tuber vermis and pyramis.

In a few monkeys the sciatic nerve was stimulated alternately with the stimulation of the cerebral cortex. In the monkey there is some spread of the cerebrocortical projection into cerebellar subdivisions which in the cat exhibit few or no responses following stimulation of the cerebral cortex. As a corollary the spinocerebellar connections are less widespread in the monkey than in the cat. In the cat it would appear that the whole of the culmen is supplied with spinocerebellar connections (Dow, 1939), while in the monkey responses to sciatic nerve stimulation are restricted to the medial parts of the lobe (Fig. 3C). The latency of the response from sciatic stimulation varied from 18 to 25 msec., as compared to 8 to 13 in the cat (Dow, 1939). It was usually a surface positive deflection.

In the monkey, as in the cat, responses were in widely separated parts of the cerebellum from stimulation of a single point on the opposite cerebral hemisphere (Fig. 3D). Conversely single lead points on the cerebellum showed potentials when the stimulus was applied over a wide area of the cerebral cortex (Fig. 4). Homolateral responses were obtained also, but they were of lower amplitude and most marked near the midline. Responses from stimulation of the most widely scattered cerebral areas were elicited when leading from the lobulus ansiformis (Fig. 4E and F, 5F).

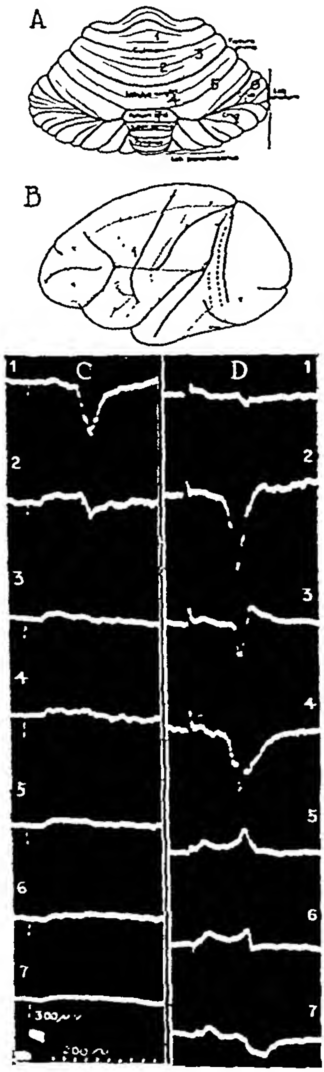
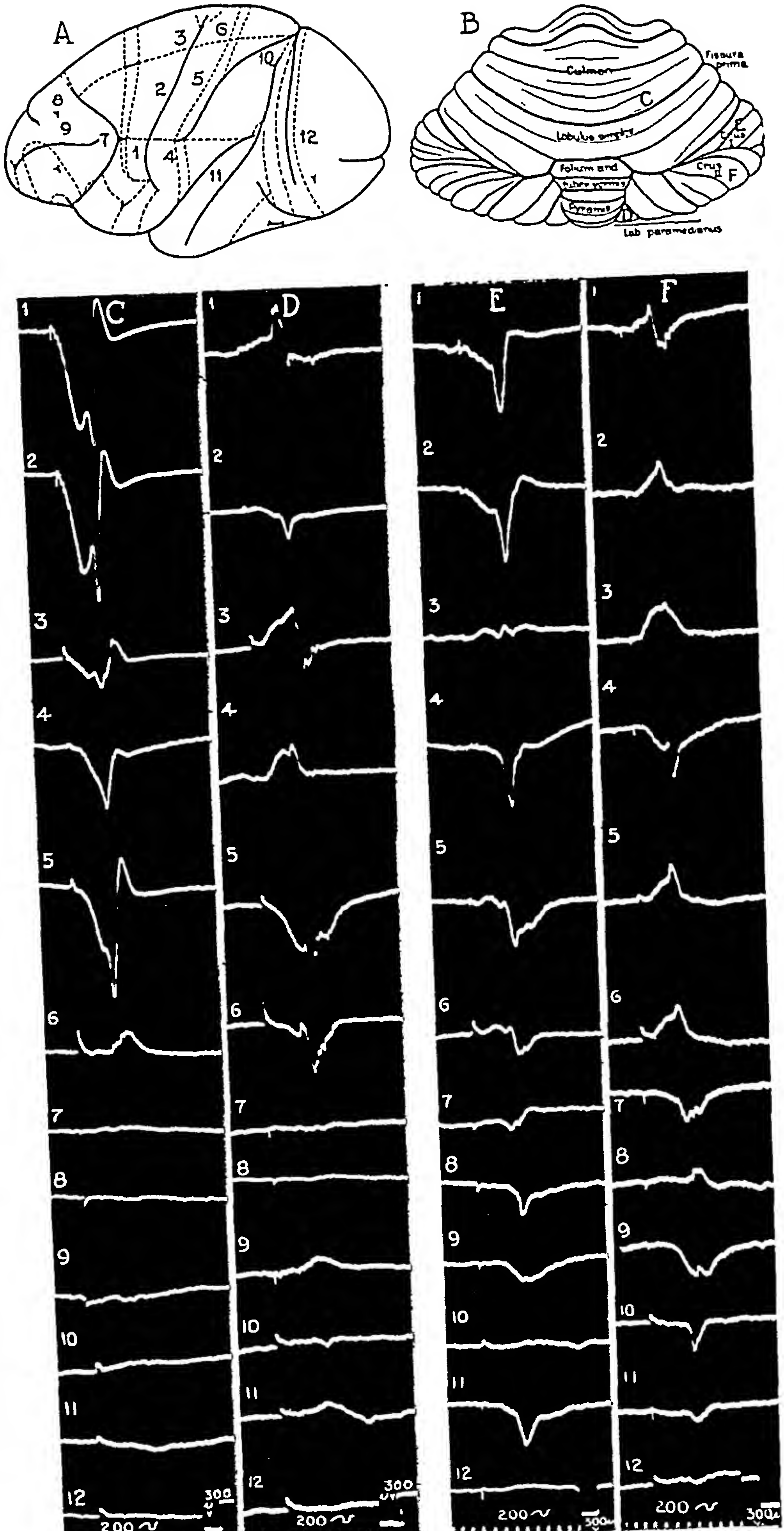


FIG. 3. A. Diagram of the anterior surface of the cerebellum of *Macaca mulatta.* Points 1 to 7 indicate the lead points for the respective records in C and D. B. Diagram of the left cerebral hemisphere of the *Macaca mulatta.* (1) Indicates the point of stimulation for the responses shown in D 1–7. C. Responses from single shock electrical stimulation of the homolateral (right) sciatic nerve. 1–7 indicate records at respective lead points as in diagram A. Note response limited to midline of culmen. D. Response from stimulation at point in arm area 4 on contralateral cortex. Note response in posterior lobe and throughout culmen, particularly in upper and lateral part.


A
B
Fissura prima
Culmen
Lobulus simplex
Crus I
Crus II
Folium and tuber vermis
Pyramis
Lob paramedianus
C
D
E
F
200 ~
300 μV

Responses when leading from the lobulus ansiformis were present following stimulation of Brodmann's areas 8, 9 and 10, areas 4 and 6, the entire parietal lobe, area 22 and occasionally areas 21, 18 and 19. In no instance were responses obtained from a stimulus at the rostral part of area 9 or in the area striata (17). The extreme frontal and occipital poles and the inferior temporal gyrus (area 20) were not explored, nor were the orbital or medial surfaces of the cortex. The areas consistently lowest in threshold responses were area 4-s and area 6 (Fig. 6).

Responses from leads on the lateral superior part of the culmen were present when the stimuli were applied to a much more restricted part of the cerebral hemisphere (Fig. 4C and 5D). In this case no action potentials were found when areas 8, 9 or 10 were stimulated. Following stimulation of areas 4, 4-s and 6, responses were elicited but those of lowest threshold were as a rule in areas 4 (Fig. 6), and those from the parietal lobe were best seen when the stimulus was near the central fissure. There were no responses from stimulation of areas 22, 21, 19, 18 or 17, and only two out of eight points stimulated in area 7 gave slight responses. Lead points lower and more medial on the culmen showed action potentials following stimulation of the same areas, but the responses were of much lower amplitude and had higher thresholds as a rule (Fig. 3D, and 5B).

Responses in the pyramis and the lobulus paramedianus were most consistent following stimulation of the precentral and postcentral gyri (Fig. 5C, E). Leads on the paramedianus and pyramis, however, showed occasional responses following stimulation of points rostral to the arcuate sulcus in the frontal association areas and in the temporal and posterior parietal lobes. These cerebellar lobules appear to occupy an intermediate position between the culmen and the lobulus ansiformis, in respect to the extent of the cortical areas having corticopontocerebellar connections demonstrable by this method.

FIG. 4. A. Diagram of the cerebral hemisphere of *Macaca mulatta*. Points 1–12 indicate stimulation points for their respective records in C, D, E and F. Records in C and D and in E and F are simultaneous potentials from the same stimulus. B. Diagram of the anterior surface of the cerebellum of *Macaca mulatta*. Points C, D, E, and F indicate lead points for records shown at C, D, E and F respectively. C. Records with a monopolar lead on the upper, lateral part of the culmen. Numbers refer to the stimulation points in diagram A. Stimulus strength 140 V. peak voltage, 0.3 msec. duration for all responses. Note absence of responses from points outside areas 4–6 and postcentral gyrus. Note the low amplitude of response from a stimulus in leg areas. D. Records with monopolar lead on the lobulus paramedianus. Numbers refer to the stimulation points shown in diagram A. Each record taken simultaneously with corresponding records in C and represent the response to the same stimulus at the two points for comparison. Note the similarity to C in the cytoarchitectonic areas giving responses but with relatively greater amplitude in the responses from leg areas. E. Lead in Crus I lobulus ansiformis. Numbers refer to the stimulation points in diagram A. Note responses 7–11 from stimuli in widespread cortical zones. F. Lead at Crus II lobulus ansiformis. Numbers refer to stimulation points in diagram A. Each record simultaneously with the record of like number in E represents the response to an identical stimulus at the two different lead points. Note absence of response in all parts of the cerebellum from point 12 in Brodman's area 17.

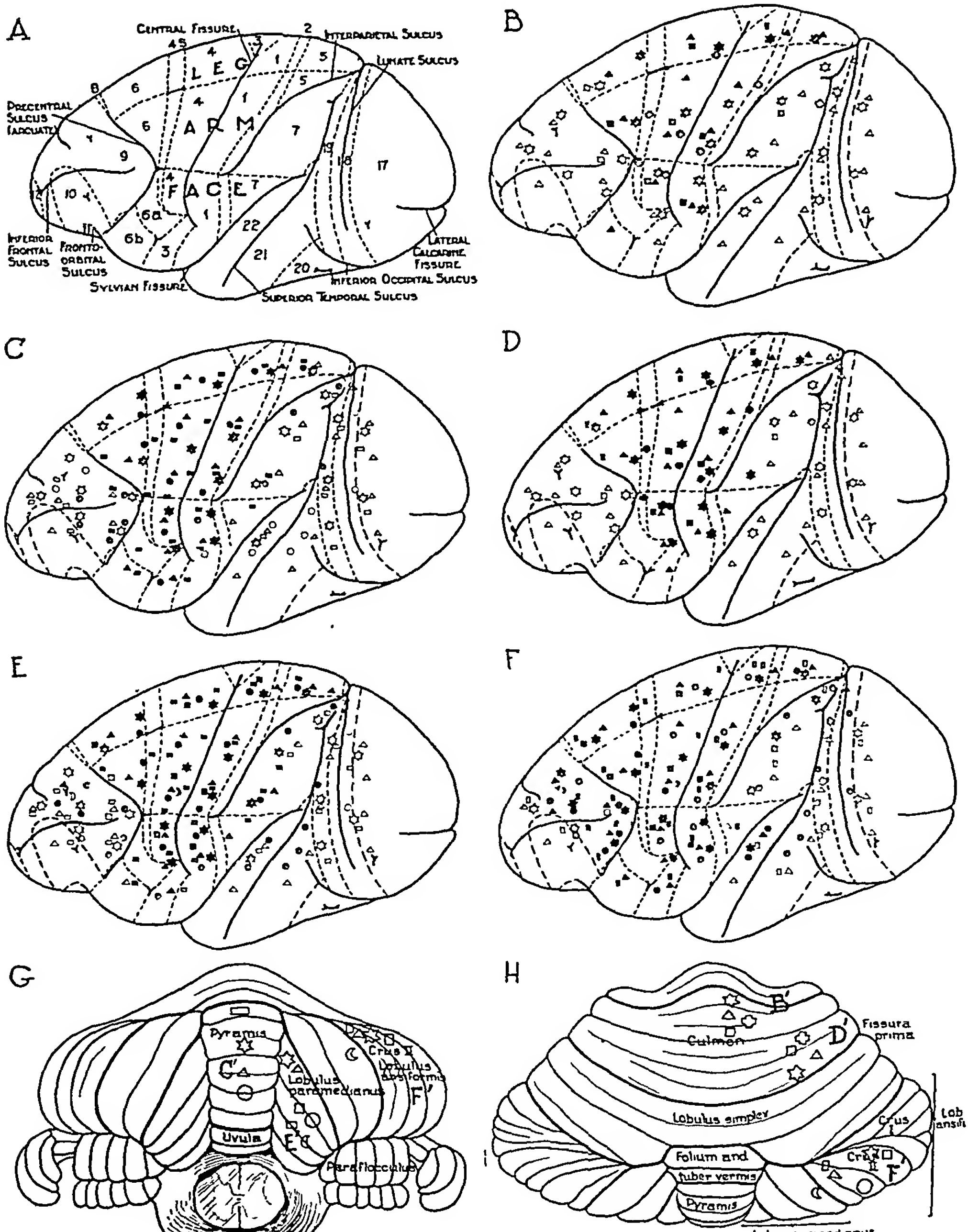


FIG. 5. A. Master diagram of the cerebral cortex of the *Macaca mulatta* showing both cyto-architectonic areas and topographical subdivisions as delineated by Dusser de Barenne, Garol and McCulloch (1941). B. Showing diagrammatically the responses at the medial lower parts of the culmen in 4 experiments. Geometric figures shown in H (B′) are lead points from which the responses were recorded. The stimulation points corresponding

Analysis of results simply on the basis of the presence or absence of responses with strong stimulation, without regard to the amplitude of the response or the threshold strength of the stimulation necessary to produce it, shows no other differences between the lobes of the cerebellum in respect to their corticopontocerebellar connections than we have already indicated. However, by giving each response recorded a rough quantitative rating on the basis of the amplitude of the response or its threshold, certain differences have come to light. These differences in threshold and in the amplitude of the response to stimuli of like strength may indicate differences in the relative richness of the projection from various parts of the cerebral cortex to various parts of the cerebellum within the pontine projection area. Figure 6 graphically depicts these differences. One may see that the projection from the face area is greatest in culmen and lobulus ansiformis Crus II, while that from the leg area is most marked in lobulus paramedianus and to a lesser degree in pyramis. It should be emphasized, however, that responses from low threshold stimulation in any topographical subdivision were obtained in all the lobes. No significant differences in the threshold of responses in Crus I and Crus II, lobulus ansiformis could be detected in any cortical subdivision, either topographical or cyto-architectonic. The percentage of low threshold responses from face and arm subdivisions was slightly higher in Crus I than in Crus II, while that from stimuli in the leg areas was slightly lower. In both lobules the percentage of low threshold responses in face areas was more than double that for either arm or leg areas.

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to each of the four lead points are represented by the same geometric figure. Each response or group of responses from that particular lead point was given a rough *quantitative rating*. No response is shown as a blank, + by a single cross hatching, + + by double cross hatching, + + + responses (*i.e.* those of lowest threshold or highest amplitude or both) by a solid block. Note the rarity of low threshold, high amplitude responses and the restriction of almost all responses to the pre- and postcentral gyri. C. Diagrammatic representation of responses at pyramis (see G) (C'). Note relatively larger proportion of + + + responses in leg area as compared to D and F. D. Same for upper lateral part of culmen (see H) (D'). Note similar total response distribution to B, but with a large proportion of + + + responses in pre- and postcentral gyri, particularly in face area. E. Same for 6 experiments with leads on lobulus paramedianus, lower part (see G) (E'). Note similarity to pyramis in the distribution of all responses, and of + + + responses. F. Same for 5 experiments with leads in Crus II lobulus ansiformis and one lead on border between lobulus paramedianus and Crus II (see G and H) (F'). Note more widespread area of responses with significant numbers of + + and + + + responses in frontal association areas, and in posterior, parietal, temporal, and peristriate areas. G. Posterior view of the cerebellum of *Macaca mulatta*. Each geometric figure indicates a lead point for a particular experiment. B' to F' indicates lead points for the stimulation points shown in the respective diagrams, B to F. H. Some as G for the anterior view of the cerebellum in *Macaca mulatta*.

It is recognized that it is impossible to place points accurately within the finer cyto-architectonic subdivisions, and errors will arise in transposing points to a master diagram such as this. However, many points in area 4 were verified at the time of the experiment by low voltage A.C. stimulation. Some points in area 4-s were identified by observing suppression effects as described by Dusser de Barenne and associates, and it is felt that, so far as the major subdivisions and gyri are concerned, the points represent the actual site of stimulation.

Figure 7C, D shows a comparison between two points within the lobulus paramedianus as originally outlined by Bolk (1906). Responses of rather high amplitude were observed at the upper point from stimulation in area 9 (Fig. 7D4). Such a response was not seen in the lower folia in this experiment (Fig. 7C4) nor in other experiments in which the lower folia of lobulus paramedianus were led from. The responses from the upper folia of lobulus paramedianus were not typical of those from lobulus ansiformis, since those of lowest threshold and highest amplitude result from stimulation of area 4 (Fig. 7D1) and not area 6 (Fig. 7D3), while the reverse is characteristic

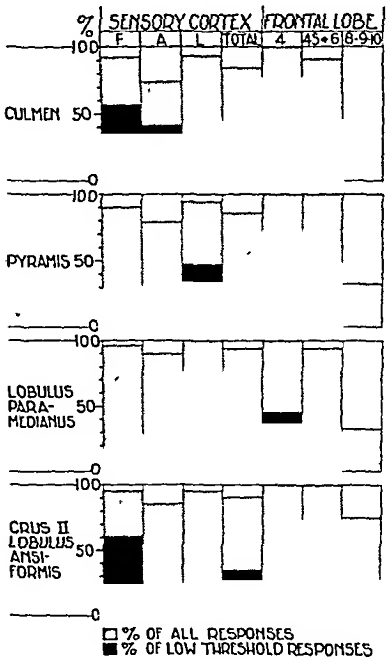

FIG. 6. Chart showing the presence and relative strength of responses from stimulation of topographical and cyto-architectonic subdivisions of the cerebral cortex when leading from culmen, pyramis, lobulus paramedianus and Crus II lobulus ansiformis. Data given in percentage of the total points stimulated in all the experiments. Note very little differences in total responses (cross hatching) except in areas 8–9–10. The solid black shows percentage of +++ responses (responses of low threshold and high amplitude). When the percentages of low threshold or high amplitude responses are compared, differences are detected. Note differences between lobulus paramedianus and lateral part of the culmen in respect to topographical subdivisions and the greater percentage of +++ responses from face than either arm or leg in the Crus II lobulus ansiformis. In comparing the various cyto-architectonic areas of the frontal lobe, the greater percentage of +++ responses in vermian lobes from stimuli in area 4, while stimuli in area 6 and the frontal association area show the greatest percentage of +++ responses in the lobulus ansiformis.

of lobulus ansiformis. There are apparently greater differences in the responses from two points within lobulus paramedianus than appear to exist between Crus I and Crus II of lobulus ansiformis, or between culmen and lobulus simplex.

The sign of cerebellar action potentials resulting from stimulation of the contralateral cerebral cortex in the monkey was not predictable. The responses were usually surface positive but might be negative or diphasic or exceedingly complex. At times a shift of the stimulation point would change completely the form and sign of the cerebellar action potential (Fig. 4F4, 5), and at other times might completely reverse the sign of the potential without altering the time course of any components. Shifting a stimulus might fail to alter the response at one lead point in any particular, while the responses from the identical stimuli were different at another lead point (Fig. 4C1, 2; D1, 2). The responses, as in the cat, seemed to separate them-

selves into those of short latency and those of somewhat longer latency. Responses of latencies approaching those of 200 msec. recorded by Curtis (1940) in the cat were not seen in the monkey, but no particular effort was made to search for them, and they may well by present in the monkey under certain conditions. The commonest response, the one most frequently elicited at threshold strength stimuli and, with rare exceptions the only type when one stimulates in the areas 8–9–10, has its peak from 18 to 30 msec. after the shock artifact. In general the greater the voltage of the action potential the shorter the latency of the response within these limits. Also it was frequently possible to demonstrate reduction of the latency of the response by several milliseconds on gradually increasing the strength of the stimulus. The other response was of much shorter latency. It too might be negative or positive in sign though most frequently the latter. It usually appeared when the stimulus was

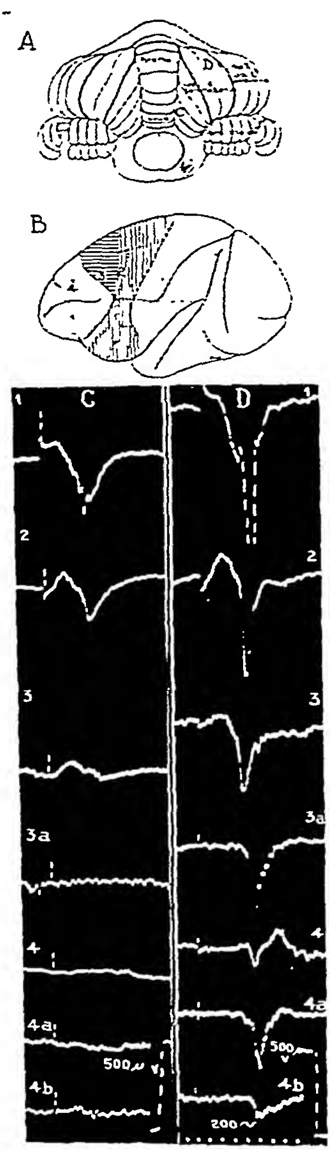

FIG. 7. A. Diagram of the posterior surface of the cerebellum of *Macaca mulatta*. Lead points for records C and D, at points C and D respectively. Note that both lie within the lobulus paramedianus as delineated by Bolk (1906). B. Diagram of the cerebral hemisphere of *Macaca mulatta*. Points 1, 2, 3 and 4 indicate sites of stimulation for records shown in C and D. Vertical cross hatching indicates area ablated before records 3a and 4a were taken. Horizontal cross hatching indicates area subsequently ablated but before record 4b was taken. Point 2 was identified as being in area 4-s by obtaining a suppression effect of Dusser de Barenne and McCulloch (1941). C. Action potentials from lead points C in lower part of lobulus paramedianus. Records 1, 2, 3 and 4 from stimulation of the respective points as shown in diagram B. 3a and 4a records from stimulation of points 3 and 4 after ablation of area 4. 4b record from stimulation at point 4 after ablation of areas 4 and 6. D. Records of action potentials recorded from a lead at point D, taken simultaneously with those shown in C. Note differences in responses from the same stimulus, particularly from stimulation at points 3 and 4 in areas 6 and 9 respectively. Note in 3a and 4a and b the continual presence of potentials after ablations as indicated in B have been made.

applied in the precentral or postcentral gyri and usually it had a higher threshold than the later appearing potential. At strong shock strengths both might be seen (Fig. 4C1, 2). The two might be the same sign, Fig. 4C2, or the reverse of one another, D3. The slope of the rising phase of these early potential is usually less steep than the later one. Its peak is frequently not

identifiable. This potential might begin as early as 3 msec. after the shock artifact. It can be picked up with bipolar needle electrodes placed on the surface of the cerebellum and undoubtedly is produced by nervous tissue in the cerebellum at the lead site. Which part of the whole potential complex is due to the afferent fibers to the folium and which is due to the activity of neurons in the cerebellar cortex is not clear. The reason for the responses of these two latencies is likewise unknown. It is presumed that both represent activity produced by corticopontocerebellar connections. Stimulation with needles in the white matter of the cerebral hemispheres did not materially affect the latencies of the responses, and both the early and late type could be seen (Fig. 1D). It was noted that when successive identical stimuli were given at the same stimulation point and the same lead point the early response was usually identical from stimulus to stimulus while the response whose peak occurred from 18 to 30 msec. was much more variable in its sign and form from stimulus to stimulus.

In one experiment responses were studied following the successive ablation of area 4 and then area 6. The responses from stimulation of area 6 are unchanged by the ablation of area 4 (Fig. 7D3a) and those from the post-central gyrus and the frontal association areas were still obtained subsequent to ablation of area 6 (Fig. 7D4a, b).

Discussion

Attempts to find any exclusive point-to-point relationship between subdivisions of the cerebral cortex and single lobules of the cerebellar cortex were unsuccessful. This was particularly true in the cat, and is in entire agreement with the observations of Curtis (1940) and in harmony with anatomical investigations of Sunderland (1940). In the monkey there seems to exist a predominance of representation from certain areas to certain cerebellar lobes. The cyto-architectonic subdivisions of the frontal lobes particularly exhibited such tendencies. Here area 4 has its richest projection to the vermian and paravermian lobes, while area 4-s and area 6 send their richest projection to lobulus ansiformis, and the frontal association areas are almost exclusively connected with the lateral lobes. Areas 7, 18 and 22 also are predominantly connected with lobulus ansiformis, while the post-central gyrus is relatively greater in its connections to culmen, pyramis and lobulus paramedianus. It is useless to speculate at this time on possibilities of the functional importance of these differences in afferent connections between vermis and hemisphere. Comparison between the topographical subdivisions of face, arm and leg areas of the so-called "sensory cortex" as mapped out by Dusser de Barenne and his associates (1938, 1941) shows a much less definite tendency for predominance to a particular lobe. However, by the use of a rough quantitative estimate of the responses by an evaluation of their threshold and the amplitude of the response, it does seem that the more rostral vermian and paravermian lobes find a larger face representation, while the more caudal lobules have the richest supply from the leg areas. This, how-

ever, is only a quantitative tendency and not in any sense an exclusive representation. Little difference could be detected between Crus I and Crus II of lobulus ansiformis, even by this rough quantitative estimate. The entire lobulus ansiformis apparently received a heavier projection from the face subdivisions than from either arm or leg subdivisions. The afferent corticocerebellar connections to Crus II lobulus ansiformis as determined by this method do not conform with the comparative anatomical data of Bolk (1906) and of Larsell and von Berthelsdorf (1941), which suggested a relationship between the Crus II and the cerebellar control of the lower extremity. This work does not disprove any such topographical relationship, but the afferent connections certainly do not suggest such topographical localization of function in the cerebellum. Furthermore, the fact that a cerebellar action potential produced by stimulation of a point in the forelimb area can be completely inhibited by a previous stimulation of a point in the hind limb area suggests that at some point there these two cerebellar projections share the same neurons. It is felt that this is probably in the pons or in the cerebellar cortex, but the effect might be the result of intracerebrocortical connections. This evidence for a common pathway between two topographically distinct areas of the cerebral cortex and one cerebellar lobe points against a topographical localization within the part of the cerebellum which is dominated by corticopontine connections.

The presence of action potentials on stimulation of the postcentral gyrus and frontal association areas after the ablation of areas 4 and 6 in an acute experiment shows that these lobes have their own corticopontine fibers, and the responses from the various cortical points are probably not dependent upon intracerebrocortical relays. A complete discussion of previously described anatomical pathways which might carry these connections is not given here, as recent contributions are covered in a current review (Dow, 1942). One might say that the finding of action potentials in the cerbellum from the stimulation of area 9 after ablation of areas 4 and 6 supports the contention of Mettler (1935) and Levin (1936) that some frontopontine fibers originate in areas rostral to area 6. One should call attention to the finding that parietal projections were much more widespread and apparently more important than temporopontine connections. Furthermore, the postcentral gyrus was apparently the richest zone of all the parietal lobe projection areas.

The lack of uniformity of the sign and form of the potentials is not unexpected in view of the great complexity of the cerebellar folial pattern, with its numerous fissures and sulci. The importance of the relation of the lead electrode to cerebellar fissures in the sign of the potentials resulting from stimulation of afferent fibers has already been reported in the case of the stimulation of the olivocerebellar fibers (Dow, 1939). It seems doubtful, even though responses from stimulation of a single cortical point are picked up over the whole corticocerebellar projection area, that all pontocerebellar fibers are activated by any one cortical stimulation, no matter how strong.

The cerebellar action potentials following stimulation in the pons (Dow, 1939) were much more uniform, but in this case probably a larger percentage of the pontocerebellar fibers were activated with each stimulus, no matter in which part of the pons the needles were placed.

Differences in the corticocerebellar and spinocerebellar connections in the cat and monkey suggest that in addition to an increase in the monkey in the size of the hemispherian lobes which have purely corticocerebellar connections, there is an invasion by corticopontocerebellar fibers of vermian lobes, which in the cat have few or none of these fibers. Coincidentally with the increase in the corticocerebellar connections in these border-line lobules there is a decrease in the spinocerebellar connections. This supports Winkler's (1923) contention that the neocerebellar connections grow into pre-existing cerebellar lobes and that the "neocerebellum" is not a separate group of cerebellar lobules superimposed upon other lobules which are exclusively paleocerebellar. These species differences in anatomical connections may also explain certain species differences in the effects of ablation of subdivisions of the cerebellum. It should make one guard against too ready transfer of data from experiments in one animal to those of another, particularly to man, where there is undoubtedly even more of an overgrowth of pontine connections than here demonstrated in the monkey.

There is no indication from this study of afferent connections that all the individual cerebellar lobules delineated by Bolk (1906) within the corticopontocerebellar projection area have functional significance. Corticocerebellar connections are not limited by these boundaries, and in certain instances there may be important differences in afferent connections within one of these subdivisions. For example, in the monkey the lateral and medial parts of the culmen are totally different in respect to spinocerebellar connections, and the upper and lower parts of the lobulus paramedianus in respect to the connections from the frontal association areas. It would appear from their afferent connections that many of these lobules defined by Bolk, convenient as they are for descriptive purposes, have little or no functional significance. A study of efferent connections may be of more significance in this regard, however, and these observations on afferent connections do not disprove a localization of function along the lines advocated by Bolk.

Summary

Single shock electrical stimulation of the cerebral cortex results in action potentials in the cerebellum in the cat and monkey. These responses recorded oscillographically are most marked contralaterally, but homolateral responses are also present. The stimulation of a single cortical point with a well localized stimulus may result in action potentials in all the cerebellar lobes which receive pontocerebellar connections.

Action potentials, in response to stimulation of the cerebral cortex in the cat, were observed in culmen, lobulus simplex, lobulus ansiformis, declive and tuber vermis, paraflocculus and pyramis. In the monkey, in addition to

these lobules, responses were present in the uvula. Border-line zones between the pontine and spinal subdivisions of the cerebellum in the cat were dominated by corticopontocerebellar connections in the monkey. It appeared from the restricted zone of spinocerebellar connections in the monkey, as compared to the cat, that this growth of pontine connections was to some degree at the expense of spinocerebellar connections.

In the cat cerebellar action potentials were elicited most consistently following stimulation of the sigmoid, coronal, anterior ectosylvian and anterior parts of the lateral, suprasylvian and middle ectosylvian gyri. There were isolated responses in occasional experiments when the posterior ectosylvian, posterior suprasylvian and posterior part of the middle ectosylvian gyri were stimulated. No action potentials were seen following stimulation of the sylvian and remaining divisions of the lateral or marginal gyri. No other areas of the cat's brain were explored in these experiments. Among the areas stimulated in the cat, no topographical or cyto-architectonic subdivision appeared to be represented exclusively or even predominantly in any cerebellar lobe.

In the monkey the most widespread cerebellar action potentials were evoked when areas 4 and 6 and the postcentral gyrus were stimulated. In addition, the stimulation of areas 8, 9 and 10 in the frontal lobe, areas 7, 18, 19, 21 and 22 in the parietal, occipital and temporal lobes, produced action potentials in the cerebellum, particularly in lateral and posterior lobules such as the lobus ansiformis. Conversely the projection, as judged by the amplitude and threshold of the responses, was relatively greater from the pre- and postcentral gyri to the vermian and paravermian lobules.

Differences in the amplitude of the action potentials and in strength of the threshold stimulus necessary to produce them were less marked when the topographical subdivisions of the cerebral cortex were compared. By comparing the percentage of low threshold points in the face, arm and leg subdivisions of the "sensory" cortex, as delineated by Dusser de Barenne and his associates, when leading from some of the cerebellar lobes, certain differences were observed which may indicate a slight preponderance of connections to certain lobes from these topographical subdivisions. Among the vermian and paravermian lobes there was a tendency for the face area to show the greatest percentage of low threshold responses in the more rostral lobes, such as culmen, while the projection from the leg zone was judged heaviest in the caudal lobules, such as lobulus paramedianus and pyramis. The arm area was intermediate between these two extremes. No differences between topographical areas could be detected if only the presence or absence of response was observed and some low threshold points to culmen were noted in the leg zone and vice versa. No significant difference in threshold or amplitude of the responses between Crus I and Crus II of lobulus ansiformis could be demonstrated when either cyto-architectonic or topographical subdivisions were compared. They both showed the largest percentage of low threshold responses from stimulation of the face areas. There was no evi-

dence from this study of afferent connections that the cerebellar control of the lower extremity rested in Crus II of the lobulus ansiformis.

The author wishes to express his thanks to Mr. Fred Claussen for technical assistance.

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THE EFFECTS OF POLARIZATION ON NERVE ACTION POTENTIALS*

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THE PROLONGED potential changes accompanying activity in polarized nerve are commonly regarded as changes in the polarization or electronic currents due to changed resistance or polarizability of the membrane (25, pp. 300–301; 28, p. 16; 10, pp. 812–814; 23, p. 715).

This explanation applied to the shorter potential changes (extrapolar decrease of polarization current and intrapolar increase of polarizing current during activity) was contested by Hermann in the early days of nerve physiology, who formulated his increment-decrement law as an alternative explanation of the experimental facts (17, 18, 19). According to this law, the modification of the polarization and polarizing currents by activity is determined by the size of the action potential wave, and this in turn is determined by the degree of polarization of the reactive membrane, not by its polarizability. The changes embodied in the increment-decrement law, increase of spike height at the anode of a constant current and decrease at the cathode, have been demonstrated beyond question in the action potential of frog A fibers by cathode ray oscillograph experiments (3, 23, 11), and the "law" is now the generally accepted interpretation of the shorter potential changes in active polarized nerves.

The inability of the "law" to explain the longer changes on the basis of the action potential as known sixty years ago was recognized by Hermann himself within ten years after his first announcement of it, and he then accepted changed polarizability as an adjunct necessary for the complete explanation of the experimental observations (20). In the light of present-day knowledge of the slower components of the action-potential, the increment-decrement law again becomes an adequate formulation of the facts, provided the negative after-potential resembles the spike in its changes of size under polarization.

In the experiments reported here, the effects of polarization on the amplitude of both the spike and the after-potentials of mammalian and amphibian A and C fibers were investigated. The effects of continuous polarization on nerve activity have been studied heretofore chiefly on frog A fibers, and the results of some of these earlier studies are quoted in this paper. Certain unmyelinated nerves (mantle nerves of molluscs, 5, 29; pike olfactory, 7) have also been investigated when polarized, and have been found to obey the

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increment-decrement law in a general way. Amphibian and mammalian C fibers have apparently not been investigated from this point of view, and the only available information on mammalian A fibers comes from a string galvanometer experiment of Verzár's (28, p. 11).

METHOD

For mammalian A fibers, the saphenous nerve of the cat was used; for mammalian C fibers, the hypogastric, vagus or other autonomic nerve of the cat. The sciatic nerve of Rana pipiens provided amphibian A fibers, the splanchnic nerve of Rana catesbiana amphibian C fibers. The action potentials of an isolated nerve mounted in a moist chamber were observed and photographed on a cathode ray oscillograph after amplification by a direct-coupled amplifier; the sweep circuit and stimuli were synchronized electrically (21). The stimuli were induction shocks from thyratron stimulators for A fibers, and induction shocks or more usually constant currents for C fibers (3–4 V., 3–5 msec. for amphibian; 2–6 V., 1–3 msec. for mammalian). The stimulus in each case was made strong enough to evoke a response from all the fibers, A or C as the case might be.

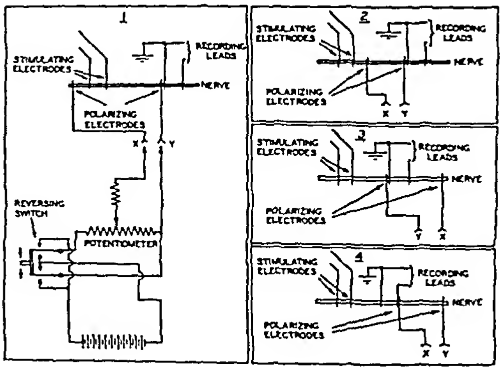

FIG. 1. Polarizing circuit and electrode arrangements. See text.

The polarizing current was applied through polarizable or nonpolarizable electrode; the pole whose effect was to be studied (the adjacent pole) was usually directly in contact with the ground lead to the amplifier, but occasionally a millimeter or so away from it on the nerve; the remote pole was 4–35 mm. (usually between 10 and 20 mm.) away. Figure 1, 1, shows the most satisfactory order of electrodes on the nerve for eliminating all possibility of unintentionally influencing the record by the effects of the remote pole; this arrangement was usually adopted with A fibers, but frequently produced unmanageably large shock artefacts with the constant current stimulation used for C fibers. Diagram 4 shows another arrangement for complete elimination of the effects from the remote pole; its disadvantage lies in interference with the early elements of the polarized (second) phase of the response by the late elements of the first phase. Diagram 3 shows a convenient but treacherous arrangement for polarization; when the remote pole is the anode, its effects are transmitted through considerable distances to the grid lead, and this introduces serious complications in the record if the response is at all diphasic. With the arrangement shown in diagram 2, the effects of the remote pole may interfere with the electrical stimulation of the nerve or the transmission of the response to the adjacent pole; with strong polarization, for instance, cathodal block at the remote pole may account for what seems to be anodal depression at the adjacent pole. Except when the polarizing current was applied as in 4, the nerve was usually crushed or heated at an appropriate point in the attempt—notably unsuccessful in the case of C fibers—to make its response monophasic. With proper precautions in interpretation, all of the above arrangements of course give comparable results.

The polarization circuit was closed by hand, and time allowed (a few seconds up to 5 min.) for the transient effects of the polarization to pass off. From the applied voltage as read on a calibrated slide wire, the fixed resistance in the circuit, and the resistance (40,000 to 200,000 Ω) of the polarized stretch of nerve measured at the end of the experiment, the voltage drop across the nerve stretch and the current could be calculated. The voltage drop so calculated was usually between 0.1 and 5 V., the current between 1 and 50 μA.; the numerical values are however of little significance, since the apparent magnitude of polarization necessary to produce similar effects in different nerves of the same kind

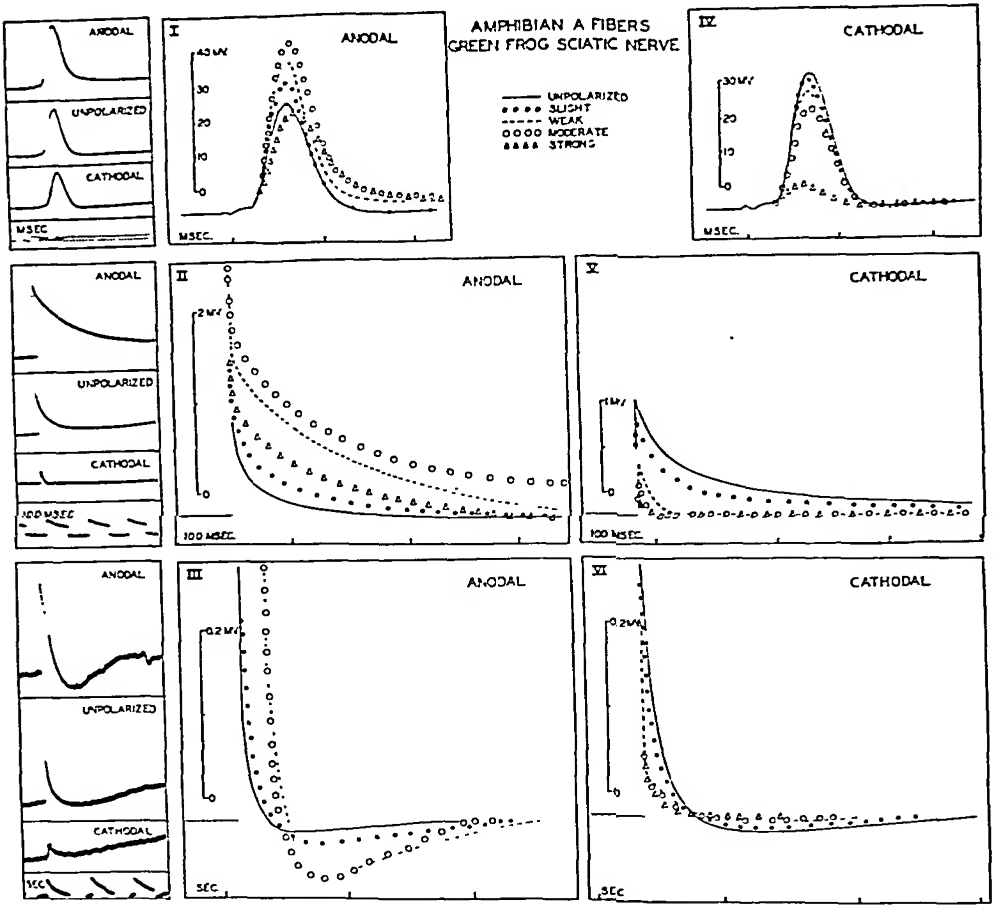

FIG. 2. Effects of polarization on amphibian A fiber action potentials. Each diagram (I–VI) consists of superimposed tracings of records taken at the indicated amplification and speed with various strengths of polarization. The effects at each pole are shown by 3 diagrams at 3 different amplifications and speeds. Actual records at zero and moderate polarization reproduced at left of each pair of diagrams. Slow tracings corrected for drift of base line seen in records. Electrode arrangement 1; interpolar distance, 28.5 mm.; interlead distance, 8 mm.; conduction distance, 13 mm. 7/5/39.

varies widely, presumably due to variation in such factors as the amount of adherent moisture and connective tissue.

A fairly rapid drift of potential frequently persisted for so long in nerves that had been polarized that it was impossible to postpone further work until its disappearance, and it therefore appears in the slow records reproduced.

Within the limits indicated below, all the effects to be described are promptly reversible.

RESULTS

A fibers. The decrease of the spike at the cathode and its increase at the anode of a constant current, already well established for the response of frog A fibers (3, 23, 11) and illustrated in Fig. 2, I and IV, is duplicated in

increment-decrement law in a general way. Amphibian and mammalian C fibers have apparently not been investigated from this point of view, and the only available information on mammalian A fibers comes from a string galvanometer experiment of Verzár's (28, p. 11).

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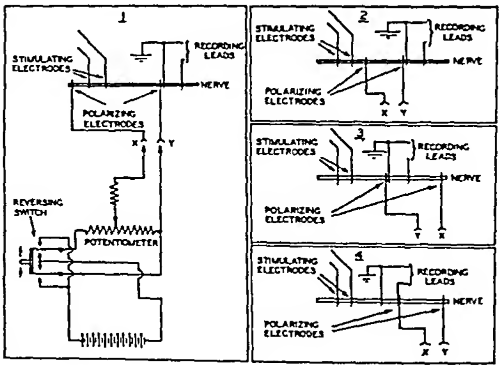

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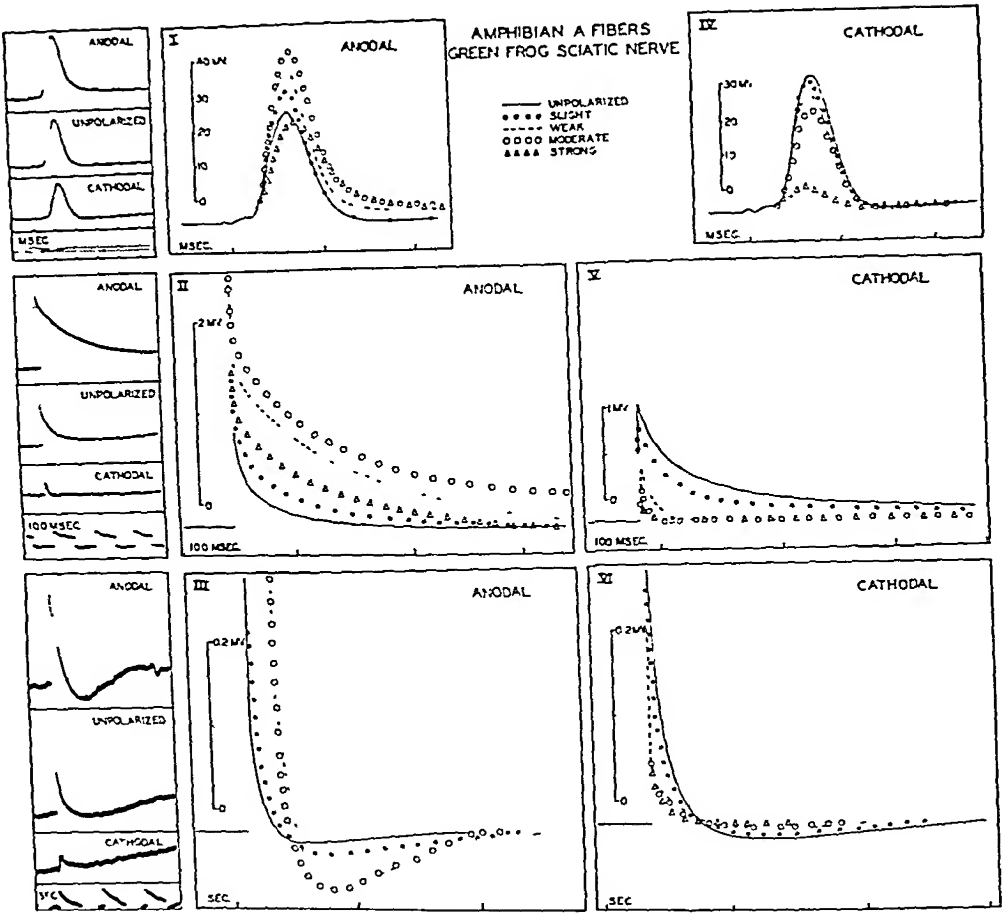

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A fibers. The decrease of the spike at the cathode and its increase at the anode of a constant current, already well established for the response of frog A fibers (3, 23, 11) and illustrated in Fig. 2, I and IV, is duplicated in

the response of cat A fibers (Fig. 3, I and IV). With either frog or cat nerve the maximal height of spike observed under anodal polarization is usually 20–40 per cent greater than the height of the spike produced by the unpolarized nerve, but the maximal increase varies from 10 per cent (or occasionally even less) to 100 per cent with individual nerves. The potential drop across the nerve for the maximal spike height is in the neighborhood of 0.5–1 V., the current 10 μA. or less. As the polarization is strengthened above this, the spike at the anode falls below its maximal height, then below normal height, and finally disappears. (Polarization of about the strength resulting in maximal spike increase at the anode will be designated as "moderate"; polarization of significantly less strength, "weak"; of still less strength, "slight." Polarization stronger than moderate will be designated as "strong," "stronger" or "extreme," according to its strength. Between any two successive steps, the voltage was usually increased 1½–2½ times.)

The fall of spike height observed at the anode with strong polarization is attributed to blocking of the response of some fibers in the nerve, since Erlanger and Blair observed with single fibers an increase at the anode up to the blocking strength of polarization, *i.e.*, no decrease preliminary to block. Their published results (11, Fig. 2, 3, 7) show an increase of somewhat over 100 per cent before block; the maximal increase of the nerve spike is thus notably less than that of the single fiber spike, probably because some of the constituent fibers of the nerve are blocked by a strength of polarization too low to induce the maximum spike in others. Greater temporal dispersion due to slower conduction at the anode (3, p. 639) may also be a factor in reducing the observed increase of the nerve spike.

With cathodal polarization of moderate strength, the spike is 40–80 per cent of its normal height, and it is greater or less than this according to the strength of the polarization. When with sufficiently strong polarization the height of the spike falls below normal at the anode, it is always still lower at the cathode (Fig. 2, I, IV; Fig. 3, I, IV), in agreement with the above interpretation of the fall at the anode.

It is known from the earlier work of others that with the increased spike height at the anode goes an increased spike duration (3, p. 645; 23, p. 716; 11); such prolongation was observed in these experiments (Fig. 2, I; Fig. 3, I), but was not investigated further.

Like the spike, the negative after-potential of A fiber responses is increased at the anode and decreased at the cathode. With both amphibian and mammalian A fibers, the increase of the negative after-potential at the anode is relatively greater than that of the spike; the after-potential may indeed be increased to several times its original size by appropriate polarization (Fig. 2, II; Fig. 3, II). The increase in amplitude of the negative after-potential with increase in strength of polarization ceases only when the polarization is strong enough to result in decreased height of spike at the anode. With weak polarization the anodally increased amplitude of the negative after-potential is usually accompanied by increased duration, especially in

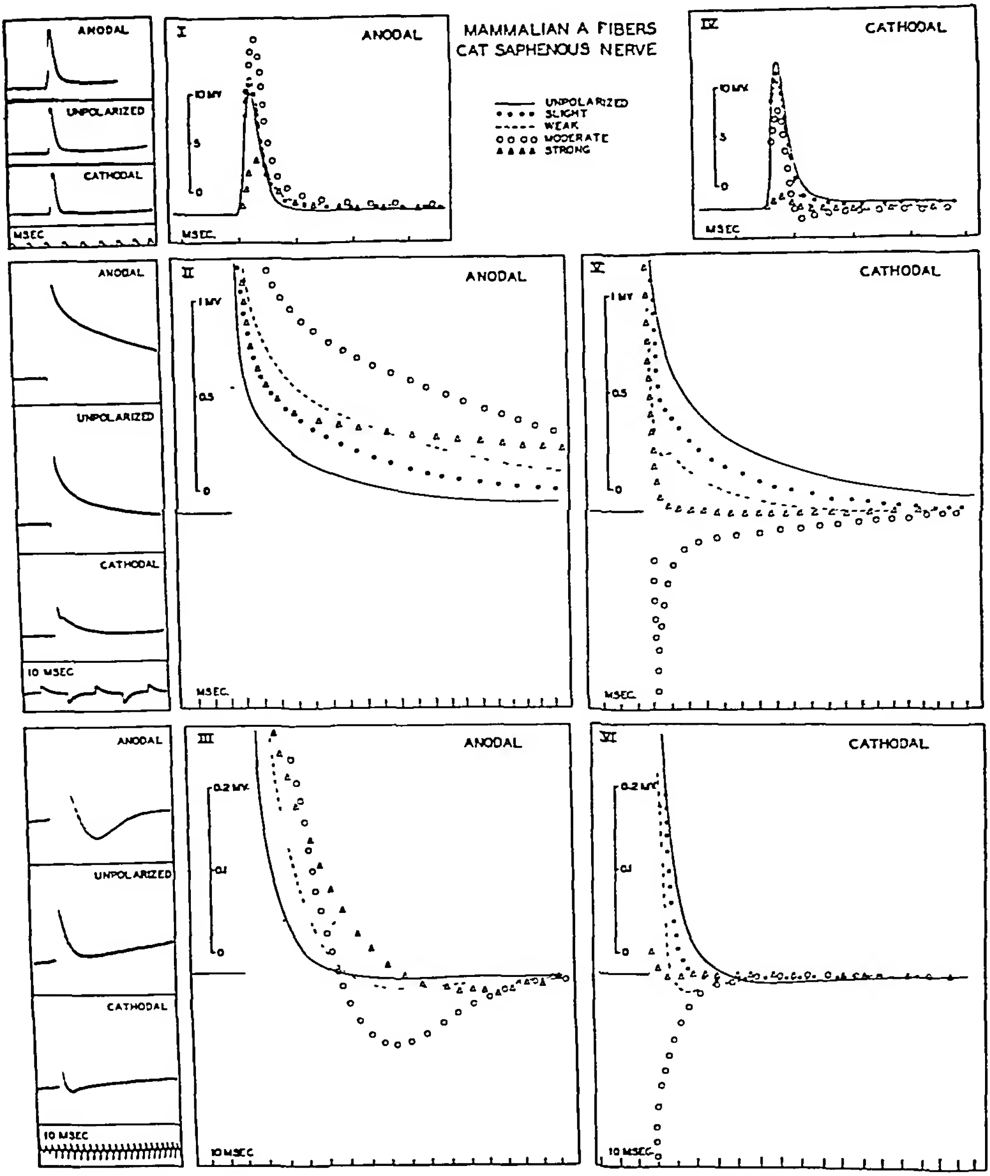

FIG. 3. Effects of polarization on mammalian A fiber action potentials. Each diagram (I–VI) consists of superimposed tracings of records taken at the indicated amplification and speed with various strengths of polarization. The effects at each pole are shown by 3 diagrams at 3 different amplifications and speeds. Actual records at zero, moderate anodal and weak cathodal polarization reproduced at left of each pair of diagrams. Slow tracings corrected for drift of base line seen in records. Electrode arrangement 3; interpolar distance, 24 mm.; interlead distance, 17.5 mm.; conduction distance, 10 mm. 5/11/39.

amphibian fibers, but as the polarization is strengthened, the development of more positive after-potential may curtail the negative after-potential. Cath-

odal polarization of moderate strength may completely suppress the negative after-potential of either amphibian or mammalian A fiber responses (Fig. 2, V; Fig. 3, V); but with some frog sciatic nerves a long low negative after-potential persists in spite of cathodal polarization strong enough to reduce the spike to a small fraction of its normal height.

The protracted negative after-potential recorded from veratrinized nerve increases at the anode and decreases at the cathode as the usual negative after-potential does (Fig. 4). The latter ordinarily manifests at the anode prolongation of its rising-phase too slight to record under the usual conditions, but the anodal prolongation of the rising-phase of the veratine after-potential is great enough to be conspicuous.

The effects of polarization on the positive after-potential are somewhat more difficult to determine because this potential is (in A fibers) relatively small, and because it varies in magnitude and duration with the condition of the fibers. It consists of two elements, P_1 and P_2, one or both of which may be absent in a single normal response (15, pp. 213–215, and references there). The presence of these two elements, overlapping in time, is revealed by a definite change in the slope of the positive potential curve under certain conditions, but the two elements can not always be so distinguished, and no such landmarks are found in the records of the polarization experiments. P_1 and P_2 can however be differentiated to some extent by their chronological position in the response. The experimental observations indicate, as shown by the following summary of them, that both P_1 and P_2 are increased at the anode and decreased at the cathode. The increase at the anode has already been referred to as interfering at times with prolongation of the negative after-potential there.

Amphibian A fibers normally exhibit relatively little after-positivity, and they do not manifest a detectable amount of P_1 under anodal polarization, perhaps because the increase of negative after-potential obscures it. The anodally increased negative after-potential converts the recorded potential from positive to negative during the interval marked by increasing positivity in the unpolarized response (Fig. 2, III), but marked P_2 develops at longer intervals, and the maximum positivity falls later than in the unpolarized response. The change of potential sign from positive to negative does not occur in mammalian A fiber responses, presumably because the development of P_1 is able to counterbalance the increased negative after-potential; it is only with polarization strong enough to result in decreased spike height at the anode that the change of sign occurs and the maximum of the positive after-potential is postponed (Fig. 3, III). The positive after-potential decreases as the negative after-potential does under anodal polarization strong enough to result in decreased spike height (Fig. 3, I, II, III, strong polarization), but quantitatively significant positive after-potential records at such strengths of polarization are hard to obtain at the anode because of a tendency to shifts of potential and "spontaneous" activity (Fig. 2, slow record at moderate anodal polarization; Fig. 3, III, end of tracing at strong

polarization). These disturbances frequently appear under moderate polarization and become greater with increasing strength of polarization; they are particularly marked after stimulation.

The characteristic decrease of the positive after-potential at the cathode is clearly seen in amphibian A fiber responses (Fig. 2, VI), because in these responses all the positive after-potential is subsequent to negative after-potential, but the decrease is obscured in mammalian A fiber responses by

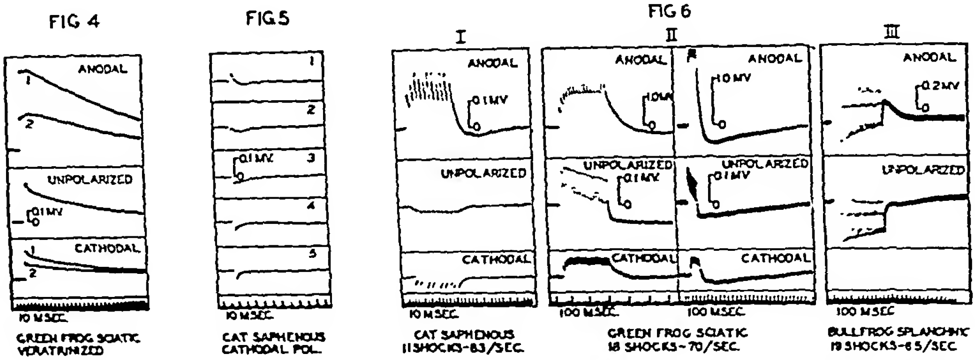

FIG. 4. Negative after-potential of veratrinized nerve polarized. Records 1, moderate polarization; records 2, stronger. All records at same amplification. Electrode arrangement 1; interpolar distance, 83 mm., interlead distance, 6 mm.; conduction distance, 9 mm. 3/21/38.

FIG. 5. Modification of early after-positivity under cathodal polarization. Record 1, unpolarized; records 2–5, weak, moderate, strong and stronger polarization. All records at same amplification. Electrode arrangement 1; interpolar distance, 51 mm.; interlead distance, 6 mm.; conduction distance, 21.5 mm. 1/30/38.

FIG. 6. Repetitive responses in nerves under moderate polarization. I. All records at same amplification. Electrode arrangement 1; interpolar distance, 51 mm.; interlead distance, 6 mm.; conduction distance 21.5 mm. 1/30/38. II. Cathodal records at same amplification as unpolarized. Electrode arrangement 1; interpolar distance, 39 mm.; interlead distance, 6 mm.; conduction distance, 22 mm. 11/14/38. III. Both records at same amplification. Electrode arrangement 3; interpolar distance, 7.5 mm.; interlead distance 3 mm.; conduction distance, 6 mm. 2/3/39a.

the exposure of P_1 (and diphasicity; Fig. 3 and 5). This exposure is the result of removal of negative after-potential which to some degree conceals the early positivity in the response of the unpolarized nerve (Fig. 3, VI, slight, weak and moderate polarization; Fig. 5, weak and moderate polarization). The positivity thus revealed is of shorter duration than the normal positivity (Fig. 5, records 1 and 2), and becomes progressively shorter as the strength of polarization is increased (Fig. 5, records 3–5). In mammalian as in amphibian A fiber responses, therefore, cathodal polarization decreases the positive after-potential.*

* Confusion arising from the negative after-potential changes accounts for the misstatements regarding the behavior of the early positive after-potential under polarization in the first account of these experiments (*Amer. J. Physiol.*, 1938, *123*: 79)

The after-potential changes under polarization produce conspicuous differences in the cumulative potentials recorded from series of responses in unpolarized and polarized nerve (Fig. 6, I and II), but repetition brings out no important new features. The negative after-potential, already large in the single response at the anode, is increased somewhat by summation with repetition. Comparison of the slow cathodal record in Fig. 6, II, with Fig. 2, VI (moderate polarization in both cases) illustrates the cumulation of positive after-potential, largely P_1 under these conditions, with repetition of amphibian A fiber responses (12, p. 41). The increase of positive after-potential under anodal polarization in Fig. 6, II, is rather less than is usually seen with these fibers stimulated repetitively.

Recovery of exitability has been followed in a few polarized nerves by the method of recording the height of response to a fixed submaximal stimulus at various intervals after a conditioning response and comparing it with the height of unconditioned response to stimuli of various strengths. The polarizing electrode was applied at or near the point of stimulation in such experiments, instead of at the recording lead. The changes in recovery observed—more rapid recovery and greater tendency to supernormality with anodal polarization, slower recovery and suppression of supernormality with cathodal polarization—are in keeping with the effects of polarization on the negative after-potential.

C fibers. As will be shown, C fiber responses are affected by polarization essentially as A responses are, but the much greater prominence of the after-potential changes in C responses makes the spike changes relatively inconspicuous. The interpretation of such potential changes as are clearly not referable to after-potential is interfered with by the temporal dispersion occurring in such slow responses even with short conduction distances, and by the great tendency of C responses to be diphasic. The spike changes are so obcured by these factors as to defy quantitative estimation and to offer no firm basis for gauging the degree of polarization; the polarization of C fibers is therefore designated "weak," "moderate," or "strong" etc. somewhat arbitrarily, though not without regard to the intensity of the effects produced and to the effects of comparable voltages and amperages in A fibers.

C fibers are less satisfactory than A fibers in polarization studies for the further reason that their responses do not readily and completely revert to the original unpolarized form after even very mild polarization. (Brodie and Halliburton, p. 185, called attention to the permanent effects of a constant current in non-medullated nerves, in contrast to medullated.) For instance, the height of the unpolarized spike varies, usually growing lower, as an after-result of either anodal or cathodal polarization; and the positive phase of the unpolarized response is frequently much smaller for an indefinite period after cathodal polarization has been applied and removed than it was originally, while it tends to be larger subsequent to anodal polarization. Nerves showing marked after-effects have been avoided as far as possible in the illustrative material used in this paper.

The presence of A fibers in nerves used for experiments on C fibers results in a shift of the start of the C fiber potential record toward the negative side with anodal polarization because of the increased negative after-potential of the A fibers (Fig. 8, I and II); when this occurs, an approximate correction may be made for it. In spite of all the difficulties, there is no doubt that the component potentials of C responses change qualitatively under polarization as do those of A responses, but quantitative estimates of the amount of change are unreliable.

Superimposed tracings of records in which the polarization effects on the spike are to a certain extent separable from those on the negative after-

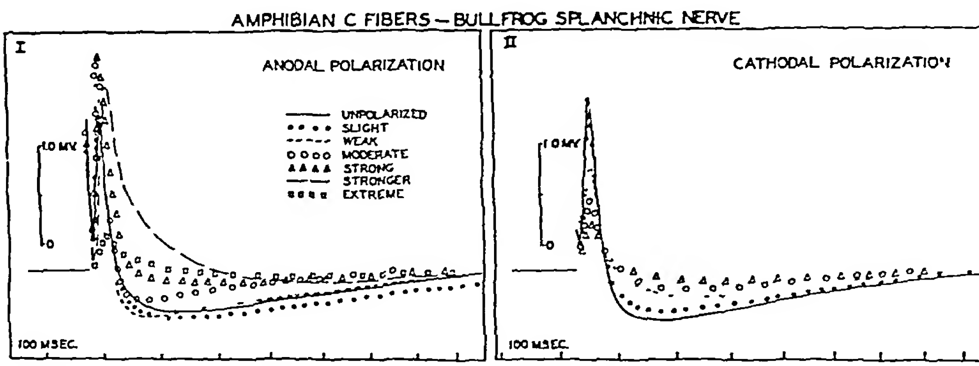

FIG. 7. Effects of polarization on C fiber action potentials. Superimposed tracings of records Electrode arrangement 2; interpolar distance, 4 mm.; interlead distance, 8 mm ; conduction distance, 6 mm. It was shown by increasing the strength of stimulation that excitability changes at the remote pole did not account for the observed changes in spike height. 4/8/39a.

potential are presented in Fig. 7, for comparison with the results in Fig. 8 in which negative after-potential obscures the behavior of the other components of the response.

In the bullfrog splanchnic nerve responses represented in Fig. 7, the height of the spike increased at the anode to a maximum (not reproduced; it occurred with a strength of polarization intermediate between strong and stronger) about 60 per cent above the unpolarized height, and fell off with stronger polarization. The amplitude of the characteristically large positive after-potential (2; 14, p. 301) increased at the anode with slight polarization, and in its early portion with weak polarization. With moderate and strong polarization, however, it was reduced in amplitude and duration at the anode; with stronger polarization it was replaced by negativity lasting more than 200 msec. With extreme anodal polarization, the whole response largely disappeared.

In the series of bullfrog splanchnic nerve responses represented in Fig. 8, II, on the other hand, there was no increased after-positivity at the anode with any strength of polarization; the whole curve shifted towards the nega-

tive side from the very start of the response even with weak polarization. With stronger and extreme polarization, the potential was recorded as one prolonged negative wave (cf. the tracings for these two strengths of polarization in Fig. 7, I). The maximum negativity reached in the experiment of Fig. 8, II, was about twice the unpolarized spike height. The fusion between the spike potential and the negative after-potential at the anode was less complete in other experiments on bullfrog C fibers than in the ones used for

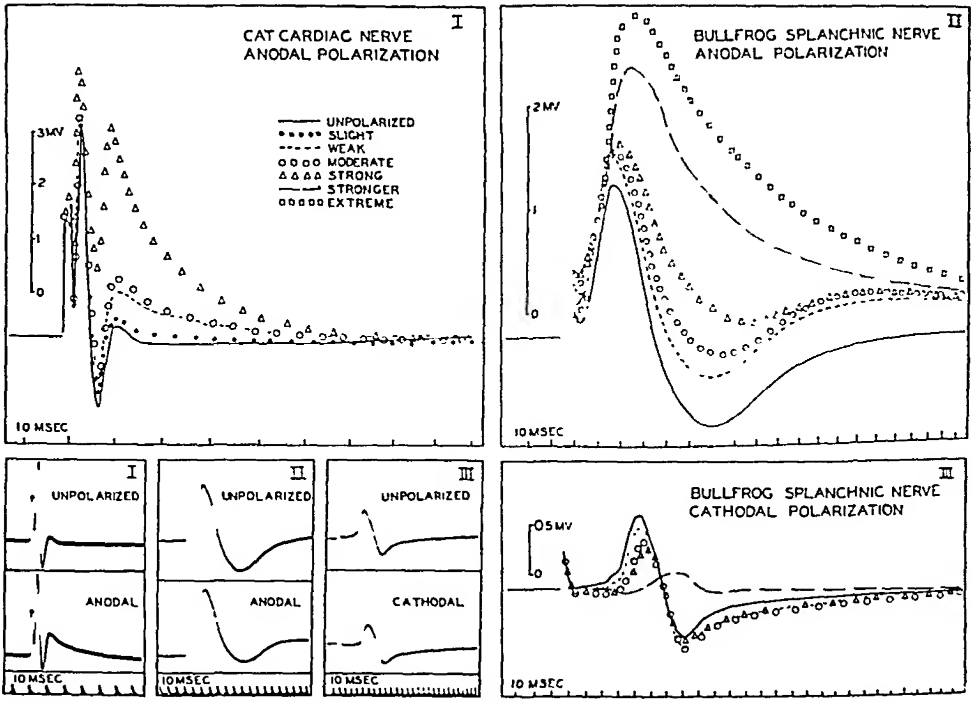

FIG. 8. Effects of polarization on C fiber action potentials. Superimposed tracings of records. Records at zero and moderate polarization reproduced. I. Electrode arrangement 3; interpolar distance, 16.5 mm.; interlead distance, 12 mm.; conduction distance, 2.5 mm. 5/11/39c. II. Electrode arrangement 3; interpolar distance, 19.5 mm.; interlead distance, 19.5 mm.; conduction distance, 4.5 mm. 4/8/39a. III. Electrode arrangement 2; interpolar distance, 11 mm.; interlead distance, 7 mm.; conduction distance, 15 mm. 3/24/39a.

the figures, and tended to be still less complete in cat C fibers; the cat C fiber responses illustrated by Fig. 8, I, show an unusually distinct separation between the spike and the negative after-potential because of diphasicity.

Are both the spike and the negative after-potential of C fiber responses, like those of A fiber responses, increased and prolonged at the anode? Convincing evidence for the increase of the constituent fiber spikes is not available from such experiments on multifibered nerves as those of Fig. 8, I and II, if it be assumed that negative after-potential from the faster C fibers develops promptly enough to raise the crest of the composite spike. Even

if this assumption be granted however, such spike increases as are accompanied by increased positive after-potential (Fig. 7, I, slight and weak polarization), must be due to actual increases in the constituent spikes. In the experiment of Fig. 7, the spike height increased to 50 per cent above its unpolarized height with polarization too weak to produce any confusing negative after-potential; this is of the order of the usual maximum increase observed at the anode with composite spikes of A fibers. Similar records of simultaneous increases of spike height and positive after-potential were obtained with cat C fibers, and it is concluded that the spikes of both amphibian and mammalian C fiber responses are increased in amplitude at the anode.

No data were obtained on the duration of C fiber spikes under polarization; is it possible that lengthened spike duration, plus the effects of temporal dispersion, accounts for all or a considerable part of the prolongation of negativity recorded? Erlanger and Blair's illustrations of single A fiber spikes show not more than doubled length at the anode before block; unless C fiber spikes differ very greatly from A in this respect, lengthened duration of the constituent single spikes can not account for the lengthening of negativity observed at the anode with C fiber nerves. The temporal dispersion of the conducted spike will increase at the anode if C fiber responses are slowed there as A fiber responses are (3, p. 639). It is clear from the records (*e.g.* Fig. 8, II) however that the slowing of conduction is not marked, and calculation based on the known range of conduction rates of bullfrog C fibers (0.6 to 0.2 m. per sec. (14, p. 299)) shows that even halving the rate will not extend spike negativity as much as 100 msec. with the conduction distances used in these experiments. Development of negative after-potential at the anode, therefore, suggested by inspection of the records as well as by the behavior of A fiber responses, probably accounts for most of the prolonged negativity observed at the anode in C fiber responses.

An example of the anodal increase of negative after-potential following repetitive stimulation of C fibers is included in Fig. 6, III; except for the reduced frequency of stimulation and the greater positive after-potential (note the positive level of potential maintained during stimulation of the unpolarized nerve) the records are similar to those of repetitive responses from A fibers (Fig. 6, I and II).

At the cathode the C fiber spike seems to decrease in height about as that of A fibers does; with the strength of polarization giving at the anode the greatest spike height to which increased negative after-potential certainly does not contribute, the height at the cathode is decreased to about 60 per cent normal (Fig. 7); this may be compared with the decrease of spike at the cathode to 40–80 per cent of normal in A fibers with polarization giving maximal spike increases at the anode. Frank negative after-potential is absent from single responses of normal bullfrog C fibers, and, as expected from the results with A fibers, is not developed at the cathode; parallel decreases of spike and positive after-potential throughout the applicable range of polarization strengths are therefore ordinarily observed with bullfrog C

fibers (Fig. 7, II), and with cat C fibers as well. Occasionally however, particularly in nerves already subjected to numerous periods of polarization, increases of after-positivity are observed with cathodal polarization (Fig. 8, III) as they are with cathodal polarization of mammalian A fiber responses (Fig. 3, VI; Fig. 5). It is believed that in the case of such C fibers, some negative after-potential overwhelmed by positive after-potential, lies concealed in the unpolarized response; the explanation of the apparent increase of positivity at the cathode is then the same as the explanation of the same phenomenon in mammalian A fibers (p. 144).

The positive after-potential in C fiber responses which has been described as increasing with the spike at the anode (until its behavior is obscured by the increase of negative after-potential) and decreasing with the spike at the cathode, is P_1; study of P_2 in polarized C fiber responses was abandoned because of difficulty in maintaining a sufficiently steady base line for the length of time required for an observation.

Discussion

Observations similar in principle to those incorporated in this report were published by Verzár in a series of papers from 1912–1928; he studied chiefly the potentials developed by frog sciatic nerves stimulated tetanically, recorded diphasically by a string galvanometer. Because cathodal polarization reversed rather than merely decreased the prolonged potentials manifested at the anode, he came to the conclusion (28, p. 16) that his observations were not explicable as prolongation of the action potential at the anode with distortion by instrumental inertia. He applied the term "depolarization wave" to the level of potential maintained during stimulation and its gradual return to the resting level following stimulation (26), because the potential was opposite in direction to the extrapolar electrotonic current. The "depolarization wave" was followed by a potential wave in the reverse direction, the positive after-potential, which Verzár correctly concluded was related to the "depolarization wave" (27). However, though he used Hering's designation of positive after-potential, he concluded that this wave was actually increased polarizability rather than positivity (27, p. 253), since both anelectrotonus and catelectrotonus were increased during its passage, and positivity would have decreased catelectrotonus.

Verzár's conception of the first prolonged potential as depolarization was adopted by Samojloff and Kisseleff, who believed they recorded it separately from the action potential on the anodal side of a polarized region when an impulse approaching the region from beyond the cathode was "blocked" at the cathode (22, p. 482; on the anodal side, "von den Aktionsströmen sieht man so gut wie gar nichts mehr"). They were unable to explain why it was not recorded when the impulse approached the polarized region from beyond the anode, although as they were then recording on the cathodal side and as usual monophasically, the explanation now seems obvious. When the "depolarization wave" appeared in records from the anodal side, the impulse actually could not have been completely blocked at the cathode.

Ignorance of the existence of the negative after-potential in unpolarized nerve, combined with (i) neglect of Hermann's warning statement (20, p. 270, footnote) that any slow potential from unpolarized nerve would balance out in diphasic recording and (ii) unintentional recording of anodally increased negative after-potential disguised as positivity at the cathode, resulted in the misinterpretations of experimental observations just cited, as in the general adoption (*e.g.* Ebbecke) of the changed polarizability view of the interaction between polarization and activity. Apparently, in the sixty years since Hermann's admission that the polar changes of the action potential known at that time were insufficient to account for the changes of polarizing and polarization currents with activity, Boruttau (4, p. 165) has been the only worker to show any hesitation at accepting the hypothetical changed polarizability. His hesitation seems to have been due to his impression that the action potential had components long enough to account for the prolonged changes of potential observed in polarized nerve (cf. 25, pp. 301–302). He expressed the opinion (5, p. 300) that the increment-decrement law of Hermann explained all the observed phenomena adequately, and while this is not true if the application of the law is restricted to the spike potential, the law is the least hypothetical and most convenient formulation of the facts when the negative after-potential is included in it. Recent indications (8 and references there; 9) of the possible number and variety of potential sources and polarizable structures in the nerve "membrane," emphasize the desirability of being as specific as may be in regard to any observed potential change. The observations reported in this paper are so readily described by the increment-decrement law that it is superfluous to do so here (see summary below, 4).

These experiments tend to confirm certain more or less well-established conclusions regarding the negative after-potential (15, pp. 214–216 and references there). (i) The negative after-potential begins early enough in the response to affect the height of the composite spike; any other interpretation of such potential forms as are illustrated in Fig. 8, II is almost impossible. (ii) The negative after-potential has a distinct rising-phase that under certain circumstances outlasts the main part of the spike (Fig. 4). (iii) Increased negative after-potential however produced is generally accompanied by shortened relative refractoriness and increased supernormality, while decreased negative after-potential is accompanied by changes of recovery of excitability in the opposite direction; polarization is evidently one of the after-potential determinants which does not disturb this relationship of after-potential to recovery (p. 144). (iv) The late positive after-potential, P_2, is a concomitant of the negative after-potential and varies in size with it in polarized nerve as in nerve subjected to other influences (Fig. 2, III, VI; Fig. 3, III, VI); this is in line with Gasser's suggestion of an intimate connection between these two potentials (13, pp. 167–169). Although not concerned with the negative after-potential, the variation in the size of the early positive after-potential, P_1, with variation in size of the spike, particularly

its increase in anodally polarized C fibers (Fig. 7, I), may be mentioned in support of the other part of Gasser's suggestion regarding the interrelationship of the component parts of the nerve action potential, *i.e.* the intimate connection between the spike and P_1.

Whatever the structures or mechanisms producing the spike potential (with its companion, P_1?) and the negative after-potential (with its companion, P_2?), the negative potentials are both reversible discharges of polarizable systems, and imposed polarization brings out certain similarities and certain dissimilarities between the two systems. They are alike (i) in being polarized in the same direction, (ii) in being approximately equally sensitive to polarization, (iii) in having their discharge time (rising-phase) prolonged when their charge is increased under anodal polarization, and (iv) in not being polarizable in the opposite direction by any strength of applied (cathodal) polarization, *i.e.* neither is ever manifest as positive potential initially. They are unlike (i) as already known, in their time relations, and (ii) in their polarizability; *i.e.*, in the degree to which their charges are increased under anodal polarization. At the anode, the spike mechanism does not at best acquire much more than twice its normal charge before it ceases to function, but the negative after-potential mechanism takes on a charge many times normal. When the after-potential mechanism fails to discharge with stimulation under polarization, it is apparently because the spike mechanism has failed, the discharge of this mechanism (the spike) being a necessary prerequisite for the discharge of the other (the negative after-potential).

Summary

1. At the anode of a constant current applied to a green frog sciatic or cat saphenous nerve (A fibers), the following changes in the amplitude of the components of the action potential are observed:

(a) The spike increases in height with increasing strength of polarization up to a maximum usually 20–40 per cent above the polarized height; if the polarization is increased further, the spike height decreases. Successive block of fibers in the nerve with increasing strength of polarization probably accounts in large part for the differences between these observations and those on single fibers.

(b) The negative after-potential increases in size up to several times its unpolarized size, and increases in duration unless it is shortened by (c).

(c) The late positive after-potential increases in size and duration in keeping with the increase in size of the negative after-potential.

2. At the cathode of a constant current applied to A fibers:

(a) The spike decreases (to 40–80 per cent of normal height with the strength of polarization giving the maximum spike at the anode); the decrease varies with the strength of polarization.

(b) The negative after-potential is decreased and may completely disappear.

(c) Early positive after-potential may appear to be increased because of (b), but is not; late positive after-potential is decreased.

3. The changes in amplitude of the various components of the action potential of C fibers (bullfrog splanchnic, cat hypogastric, vagus or other autonomic nerve) are in general the same as those just described for A fibers, but the after-potential changes are relatively greater. Under favorable circumstances, increase of early after-positivity with anodal and decrease with cathodal polarization may be observed.

4. These observations may be simply and satisfactorily described by the statements: (i) the negative after-potential, like the spike, is increased in amplitude at the anode and decreased at the cathode; and (ii) the positive after-potentials are affected by polarization as are the negative potentials which they succeed.

5. These observations are consistent with already known properties of the components of the action potential.

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STIMULATION OF PERIPHERAL NERVE TERMINATIONS BY ACTIVE MUSCLE

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A SINGLE shock applied to the distal segment of a severed ventral root results in the appearance of a group of impulses which reflect from the periphery into a fraction of the stimulated ventral root fibers, into unstimulated but neighboring ventral root fibers, and into fibers of the corresponding dorsal roots (9). Centripetal discharges have been observed in ventral roots or motor nerves of eserinized preparations (10, 4, 6, 5) These centripetal discharges have been regarded as arising at the motor nerve endings (under the influence of eserine) and coursing both orthodromically into the muscle there to cause the repetitive responses characteristic of eserinized muscle (3) and antidromically along the motor nerve (ventral root) where they may be recorded. Two lines of evidence stand contrary to this view. Eccles, Katz and Kuffler, (5) point out that the association of "repetitive" motor nerve responses with repetitive muscle response is not invariable. Secondly the centripetal responses have been observed in non-eserinized preparations, and in root fibers other than those occupied by the efferent volley to the muscles.

Afferent activity in dorsal roots would naturally ensue upon active contraction of the muscles (12), but the dorsal root activity at present under consideration does not possess the features to be expected in the case of discharges from any known tension receptors.

It is the purpose of the present study to show the origin and nature of the secondary centripetal discharges in the normal (*i.e.* non-eserinized) preparation. Some of the pertinent observations have been presented briefly in a preliminary note (9).

The animals used in these experiments were cats, and two rabbits, one of which showed characteristic secondary centripetal activity. According to Feng and Li (6) normal "after discharge" occurs also in rats. The animals were narcotized with Dial (0.5 ml. per kg.). Selected dorsal and ventral roots were exposed by laminectomy and severed from their connection with the spinal cord for the purpose of placing stimulating and recording electrodes.

When the seventh lumbar ventral root (L.7 V.R.) is stimulated by a single maximal shock, recording leads on that root reveal the spike potential of the stimulated volley (the primary motor volley) followed, after some 2.5 msec., by an asynchronous discharge lasting, on the average, ca. 5 msec. (Fig. 1A). An essentially similar result is obtained if the first sacral ventral root (S.1 V.R.) is substituted (Fig. 1C). The records of Fig. 1 were all obtained with one of the recording leads at the killed end of the root in question. When both recording leads are placed on the intact root distal to the

stimulating electrodes the phases of the potentials of the primary motor volley and of the secondary discharges are reversed indicating clearly that the primary motor volley passes distally to the muscles, whereas the secondary impulses are centripetal (cf. Lloyd, 9, Fig. 1F, L, M).

The discharge of 1 B of Fig. 1 is recorded from the S.1 V.R., and results from a single shock delivered to the L.7 V.R. The primary motor volley is of course not recorded. The secondary centripetal impulses are present, although the discharge is not as intense as it is in the L.7 V.R. The latency of

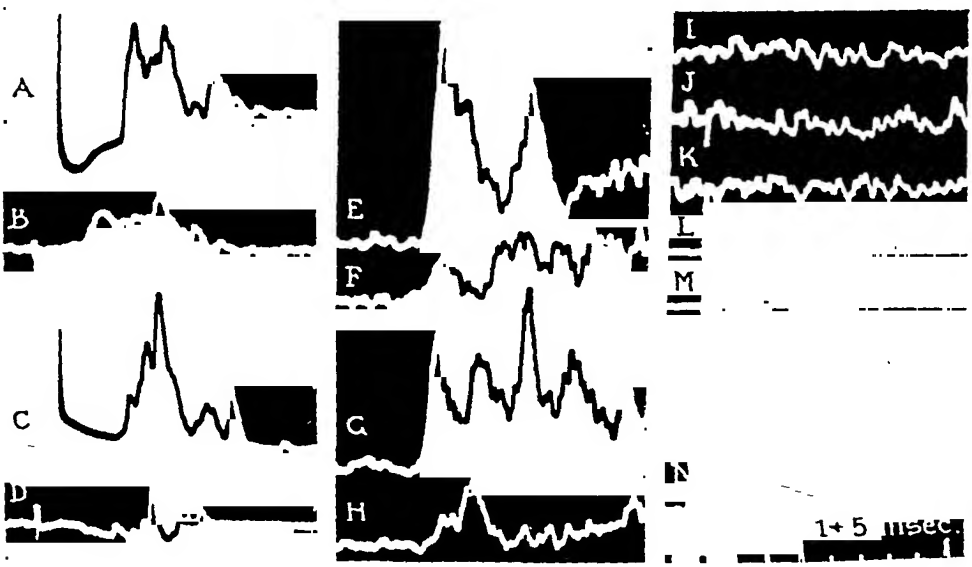

FIG. 1. Distribution of secondary centripetal discharges in ventral and dorsal roots following stimulation of ventral roots. Time in 1 and 5 msec. divisions below record N. In all figures where there are two time designations, these are for the small and large divisions respectively. Further description in text.

the secondary centripetal discharge is shorter in 1B than in 1A or 1C, being ca, 1.6 msec., as compared with 2.5 msec. The differential is not always so great, although it is generally present unless other factors are operative. Figure 1D shows the secondary centripetal discharge in the L.7 V.R. resulting from a S.1 V.R. shock.

From a consideration of Fig. 1 (A to D) it becomes apparent that the occurrence of secondary centripetal discharges in ventral roots does not depend upon the identity of the nerve structures transmitting the primary motor volley and the secondary centripetal discharges. Secondary centripetal discharges, on the other hand, are not encountered if several segments intervene between the "donor" and "recipient" ventral roots. The spatial limits to the distribution of the secondary centripetal discharges appear, therefore, to be set by the extent of spatial fusion in the distribution of the several ventral roots to the muscles. Spatial fusion is extensive in the

limb musculature (16), which no doubt accounts for the substantial secondary centripetal discharges encountered in ventral roots adjacent to a root occupied by the primary motor volley. Inasmuch as the secondary centripetal discharges do occur in roots not occupied by the primary motor volley, they are not repetitive discharges with respect to that volley.

Secondary centripetal impulses occur in corresponding dorsal roots with comparable latency. Figure 1 (E, F) shows these impulses recorded from the seventh lumbar dorsal root (L.7 D.R.) and the first sacral dorsal root (S.1 D.R.) following single shock stimulation of the L.7 V.R. Similarly Fig. 1 (G), H shows secondary centripetal responses in the S.1 D.R. and L.7 D.R. to stimulation of the S.1 V.R. The earliest dorsal root secondary centripetal responses occur usually after 1.6–1.7 msec. latency. Again, as in the case of the ventral root secondary centripetal responses, it will be noted that the dorsal root secondary centripetal responses are greatest when the ventral root of the same segment is stimulated.

Figure 1 (J to N) illustrates the ineffectiveness of centrifugal volleys in dorsal roots with respect to the initiation of secondary centripetal discharges. In 1J, the L.7 D.R. is stimulated while recording from the S.1 D.R. In 1K, stimulating and recording leads are reversed. The activity recorded in J and K is similar to that recorded in I without stimulation, and represents the normal and constant flow of afferent activity over the dorsal roots (1). For records L and M recording leads were placed on the L.7 V.R. and S.1 V.R. respectively, in each case the dorsal root of the same segment being stimulated. Again no secondary centripetal activity results. Finally in N, both stimulating and recording electrodes were placed on the S.1 D.R. A primary spike potential is recorded unaccompanied, however, by any sign of secondary centripetal activity.

It is clear from the observations of Fig. 1 that secondary centripetal discharges result only when a ventral root is stimulated and occur in fibers, both dorsal and ventral root, having a degree of anatomical fusion of peripheral distribution.

It has been noted that the latency for secondary centripetal discharge in a stimulated ventral root is longer than that in unstimulated ventral roots or in dorsal roots. Differences other than latency exist between the secondary centripetal discharges of stimulated and unstimulated roots. These are: (i) the relative size of those discharges, and (ii) the size of the primary motor volley necessary to procure threshold secondary centripetal discharges in dorsal and stimulated ventral root fibers.

Figure 2 presents a graph relating the amplitudes of the dorsal root and stimulated ventral root secondary centripetal discharges, plotted in similar arbitrary units but on different scales, to the size of the primary ventral root volley expressed in per cent of maximum. The dorsal root secondary centripetal discharge is much greater than that of the ventral root. This fact may be appreciated also in Fig. 1, 3 and 4 by noting that the ventral root responses have been recorded regularly at an amplification five times that

used for the dorsal root responses. Figure 3 further indicates the much lower threshold for production of the dorsal root secondary centripetal responses than for the production of the ventral root secondary centripetal responses.

Thus there are differences in latency, size and threshold to distinguish between the secondary centripetal discharges in dorsal and stimulated ventral roots. These differences are consentient in indicating that the motor fibers, as a result of the passage of the primary motor volley, are partially refractory to the excitatory process causing the secondary centripetal discharges.

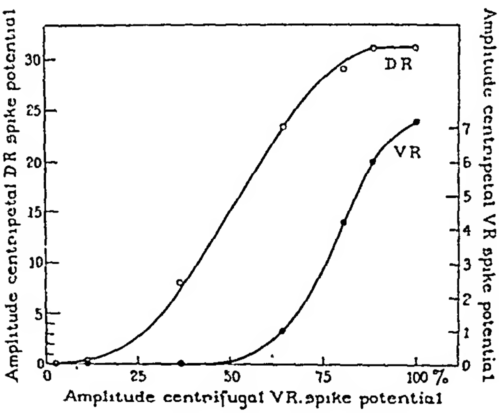

FIG. 2. Graph relating the size and threshold of secondary centripetal discharges in a dorsal root and a stimulated ventral root. The first spike potential of the discharges was selected for measurement and illustration merely because some part of the complex discharge must be chosen. Other parts of the secondary centripetal discharges respond similarly to ventral root shocks of increasing intensity.

Elements of the secondary centripetal discharge. It has been shown that the secondary centripetal discharge is asynchronous, having a duration of ca. 5 msec. This could be due to repetitiveness, or to a variety of conduction times occasioned by circuits of various lengths. To decide between these two alternatives, advantage is taken of the fact that the constituents of the lumbosacral cord break up into three major groups; the gluteal and other nerves innervating muscles about the hip, the hamstring group to muscles of the thigh, and the sciatic group to muscles of the leg. These groups are characterized by different average conduction distances between the spinal roots and the muscles concerned; each group may be interrupted in isolation from the others. The results of experiments in which selective denervation of these muscle groups was practised are illustrated in Fig. 3.

Figure 3 (A, E, I) shows the ventral root (L.7) secondary centripetal discharges in three experiments preceding surgical intervention in the leg. Similarly Fig. 3 (C, G, K) shows the dorsal root (also L.7) secondary centripetal discharges in the same three experiments and under the same conditions. Denervation of hip muscles changed the ventral root secondary centripetal discharge from that recorded in 3A to that recorded in 3B, and the dorsal root secondary centripetal discharge from that in 3C to that in 3D. The initial segment of the secondary centripetal discharge is depleted in each case;* the terminal segment is retained.

* The spike potential indicated by an arrow in Fig. 3D serves to illustrate one of the sources of confusion inherent in denervation experiments. This spike potential is not present in the normal record 3C. It has appeared *de novo* as a result of nerve section. Its latency is ca. 0.5 msec. shorter than that of the first activity in 3C. It may be identified

When the hamstring nerves are severed the secondary centripetal discharge in ventral roots is changed from that recorded in 3E to that recorded in 3F, while the dorsal root secondary centripetal discharge is changed from that in 3G to that in 3H. Figure 3 (E to H) shows that the intermediate segment of the secondary centripetal discharge is obliterated by denervation of the hamstring group. In similar fashion the observations 3I to 3L

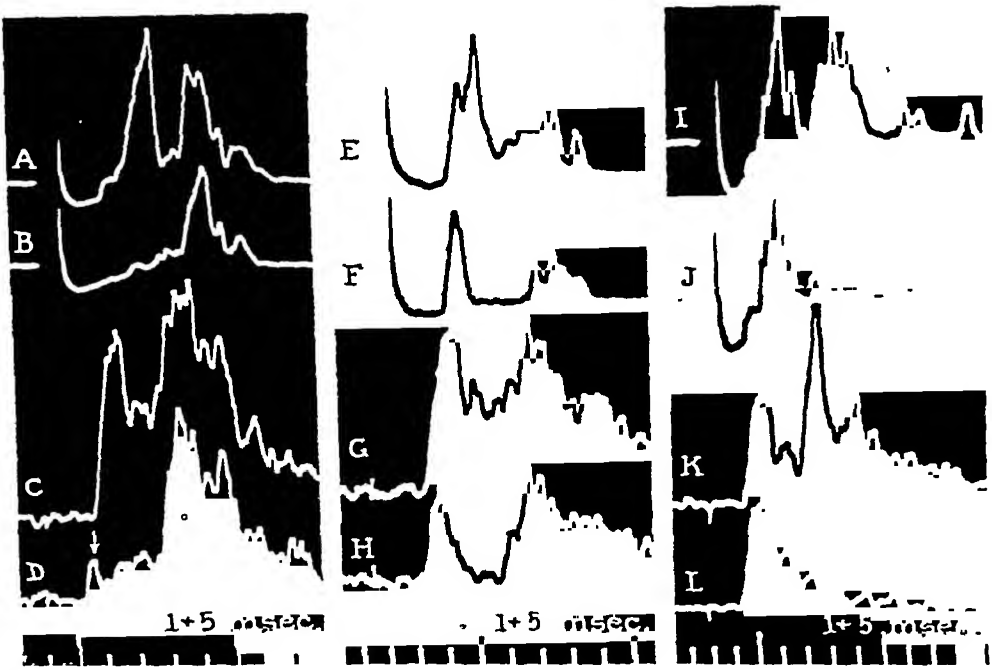

FIG. 3. Removal of specific segments of dorsal and ventral root secondary centripetal discharges by selective denervation of muscle groups. The spike potential introduced in record D after nerve section is a "denervation artefact."

show that the terminal portion of the secondary centripetal discharge is removed by section of the sciatic nerve (compare J with I and L with K), whereas the initial portion is retained almost perfectly intact.

There can be little doubt that the significant factor for dispersion of the secondary centripetal discharge is variety of conduction distance to and from the several groups of nerve endings, efferent and afferent, and that the latency of each segment of the discharge is related to the total conduction distance involved. It will be noted that the major discharges of any particular group remaining intact occur with a shorter latency in the dorsal roots than in the stimulated ventral roots. The initiation of the later segments of

as due to stimulation of D.R. fibers at the cut end of the common nerve trunk by closely intermingled active V.R. fibers (cf. 7). Another source of error arises in the stimulation of cut nerve ends by active muscle (11). Careful preparation usually reduces these "artefacts" to insignificance.

the secondary centripetal discharge in the stimulated ventral root therefore appears to be subject to the same conditions of partial refractoriness that hold for the initiation of the shortest latency secondary centripetal discharges (cf. Fig. 1 and 2). The latency differential between dorsal and stimulated ventral root secondary centripetal discharges would necessarily hold for each segment of those discharges if the secondary centripetal discharges are initiated in the terminal regions of "recipient" fibers at a relatively fixed time with respect to the arrival of the primary motor volley at the various motor fiber terminations, conduction distance being the major variable.

In occasional experiments one or two centripetal discharges occur in a few fibers after the completion of the major secondary centripetal discharge (cf. for example Fig. 3I). It is possible that these atypical discharges are repetitive in nature.

Factors contributing to the latency of secondary centripetal discharges. Nerve conduction time is a significant factor in latency of secondary centripetal discharges, but nerve conduction alone cannot account in full for the latency of even the earliest of these discharges for, as seen in column 7 of Table 1, the latency of secondary centripetal discharges is 0.54 to 0.8 msec. longer than that of "Hering phenomena" (stimulation of nerve by nerve) conducted over nerve paths of comparable length. The discrepancy between the latency of secondary centripetal discharge and the Hering phenomenon is also seen in Fig. 3(C, D). The exclusion of nerve stimulation by nerve as an explanation of the secondary centripetal discharges does not rest upon the evidence of differential latency alone. The fact that secondary centripetal discharges reflect into a *maximally* stimulated "donor" ventral root (Fig. 1, 3 and 4), whereas Hering phenomena do not is a powerful argument against

Table 1

Nerve-muscle group 1	Time from stimulus to muscle action potential 2	Conduction time, muscle to root 3	Sum of 2 and 3 4	Latency of secondary centripetal discharge 5	Latency of Hering phenomenon on sciatic N. at equivalent conduction distances 6	Excess over over 6 7
Hip*	1.1	0.5	1.6	1.64	1.1 (10 cm.)	0.54–0.56
	1.15	0.45	1.6	1.66†		
Hamstring	1.5	0.76	2.26	2.3	1.76 (16 cm.)	0.54
Sciatic	2.0	1.4	3.4	3.5‡	2.7 (24 cm.)	0.8

* This experiment is illustrated in the preliminary paper (Lloyd, 9, Fig. 1B, C, D, E).

† This value was obtained at the beginning of the experiment. When the active muscle field was restricted by nerve section a denervation artefact appeared with shorter latency, obscuring the normal onset of the secondary centripetal discharge.

‡ The records from which this figure was obtained contain a persistent denervation artefact emanating from the hip region. This artefact, however, did not encroach upon the secondary centripetal discharge.

an analogy between the two phenomena. Accordingly the stimulating agent causing the secondary centripetal discharges and that causing the Hering phenomenon may not be homologized for the former outlasts the absolutely refractory period of the nerve fibers carrying the primary motor volley with an intensity sufficient to reexcite those same fibers during partial refractoriness. In the case of the Hering phenomenon the stimulating agent is coincident with the arrival of the donor nerve volley (13) and does not persist long enough to clear refractoriness of the donor nerve fibers. The observations presented in Table 1 indicate that it is the time dissipated in neuromuscular delay before the stimulating agent causing the secondary centripetal discharges begins to act that allows the agent to clear the refractoriness of the nerve fibers occupied by the primary motor volley.

Table 1 shows the results of experiments designed to account for the duration of the latent period of the secondary centripetal discharges and to locate the time at which these discharges are initiated at the periphery in relation to the time sequence of events following upon a ventral root shock. A comparison of the figures in columns 4 and 5 indicates that the latency of a selected segment of the secondary centripetal discharge is approximately equal to the sum of conduction time to the muscle, neuromuscular delay and conduction from the muscle to the root recording leads. The residual time (0.04–0.1 msec.) may be accounted for as utilization time. The earliest secondary centripetal discharges are initiated, therefore, shortly after the onset of the muscle action potential at the junctional region.

Although the total secondary centripetal discharge in the intact leg lasts for ca. 5 msec., the discharge from a restricted muscular field is much shorter (Fig. 3). Restriction of the donor muscular field by denervation (Fig. 3 and Lloyd, 9, Fig. 1B) or by the use of threshold responses (for example in Fig. 6) shows that the unit secondary centripetal discharge has a total duration of 1 to 1.3 msec. at the recording leads. Allowing for the duration of the individual nerve spikes in the secondary centripetal discharge, but not for conduction dispersion, it appears that the last secondary centripetal discharges of a homogeneous group are initiated in the nerve endings within ca. 0.5 to 0.8 msec. of the onset of the first of such discharges. This time coincides approximately with the crest time of the muscle action potential. The secondary centripetal discharges are initiated, therefore, by processes approximately equal in duration to and contemporaneous with the ascending phase of the muscle action potential at or near the junctional region.

Contributary evidence for the participation of neuromuscular transmission and muscle action in the sequence of events leading to secondary centripetal discharges. If muscle action is a step in the production of secondary centripetal discharges then these discharges should be susceptible to modification in a predictable manner by agents and procedures known to influence neuromuscular transmission and muscle action.

(*1*) *The action of curare.* Curare removes the secondary centripetal discharges even when administered in doses which are not quite sufficient for

the complete blocking of muscular response. Figure 4 (A, E, I) shows the secondary centripetal discharges in L.7 V.R., S.1 V.R. and L.7 D.R. in response to a L.7 V.R. shock. Figure 4 (B, F, J) recorded after the administration of curare, illustrates the removal of the secondary centripetal discharges in each case. Figure 4J is of interest for it reveals a response of recruiting nature and of much longer latency still present after a degree of curarization sufficient to remove the secondary centripetal discharges in the dorsal root and elsewhere. Positive identification is not easy, but it is most

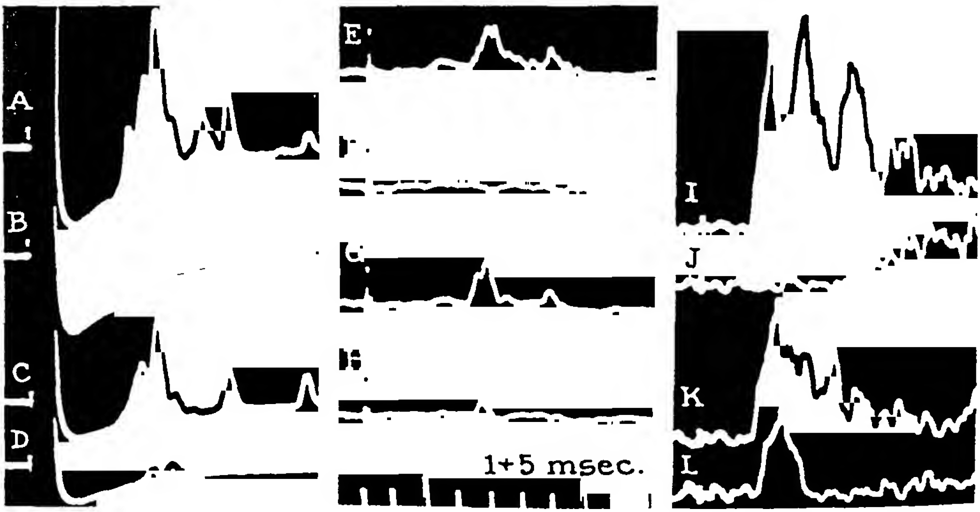

FIG. 4. Removal of secondary centripetal discharges by curare. Inhibition of secondary centripetal discharges by the Wedensky effect at the neuromuscular junction of partially curarized muscle.

likely that this response represents afferent activity initiated by the residual muscle contraction acting on tension receptors.

One might suppose that the secondary centripetal discharges should disappear only with the advent of complete curarization. Reference to Fig. 2, however, shows that the first (and lowest threshold) secondary centripetal responses appear as a result of primary motor volleys between 12 and 35 per cent of maximum size. Asynchronous recruiting afferent activity, comparable with that in Fig. 4J, appears following near threshold motor volleys. Differential threshold then is adequate to account for the segregation by curare of later afferent activity from the secondary centripetal discharge.

With small doses of curare the secondary centripetal activity is present, with latency unaltered, but with intensity diminished (compare Fig. 4C, G, K with the control observations 4A, E, I). If two shocks are delivered in succession to a ventral root of a partially curarized preparation at intervals up to 5 or more sec., the secondary centripetal activity elicited by the second of such pairs of shocks is markedly reduced. The pairs of responses

C and D, G and H, and K and L were recorded at an interval of 1 sec. in each case. The reduction in the secondary centripetal activity elicited by the second shock of each pair is readily apparent. The effect is clearly a reflection of the prolonged "Wedensky type" inhibition characteristic of partially curarized muscle (8, 2, 15).

(2) *The action of eserine.* It has been known for some time that the response of eserinized muscle to a single motor volley is repetitive (3). Likewise it has been in the eserinized preparation that repetitive centripetal activity has been observed (10, 6, 5). It remains to be shown, however, that the repetitive nerve activity is primarily grafted onto the secondary centripetal activity present before the administration of eserine. Figure 5 illustrates the secondary centripetal discharges evoked in one half of the L.7 V.R. by stimulation of the other half before (A) and after (B and C) the administration of eserine (0.5 mg. intravenous). Comparison of 5A and 5B reveals that the original secondary centripetal discharge group is retained and that the repetitive discharge is added thereto. Figure 5C, similar to 5B but recorded on a slower time axis, illustrates how the repetitive discharge is prolonged in time.

It is difficult in view of the other evidence presented here to escape the conclusion that the repetitive centripetal discharges recorded under the influence of eserine result from the repetitive response of the muscles to a single nerve volley, rather than *vice versa* as proposed by Masland and Wigton. The present interpretation has the additional merit of accommodating the observation of Eccles, Katz and Kuffler (5) that repetitive muscle responses caused by eserine frequently occur without discharges into the motor nerve. The explanation of the variable association would lie in the intensity of the muscle action necessary to secure centripetal discharges (cf. discussion in connection with Fig. 2 and 4).

The occurrence of centripetal discharges associated with the spontaneous fasciculation of eserinized cat's muscle, first observed by Masland and Wigton, has been confirmed.

(3) *The stages of neuromuscular transmission.* One of the prominent characteristics of neuromuscular transmission is the series of variations in muscle response to maintained tetanic stimulation of the motor nerve. These variations have recently been systematized by Rosenblueth and coworkers (14) into five stages of neuromuscular transmission. The influence of these stages of neuromuscular transmission on the initiation of secondary centripetal discharges has been observed, in varying degree, in all the preparations made for that purpose. The second stage is often absent, but the stages representing "treppe" and "fatigue" are regularly present. The optimal preparation for observing the treppe phases (stages 1 and 3) is that employing separate ventral roots for stimulating and recording.

Observations D to K of Fig. 5 illustrate the influence of the initial stages of neuromuscular transmission on secondary centripetal discharges and were recorded in the following manner. A single response (5D) was recorded.

The succeeding responses were recorded at intervals as a standing wave, the stimulus being synchronized with the sweep, the stimulus frequency approximating 50 per sec. There are slight variations in individual responses in addition to the slower trends; hence the double exposure effect in records E to K. In the experiment illustrated the second stage is present resulting in an initial decrease in the secondary centripetal discharge, succeeded by an increase (third stage) and "fatigue" (fourth stage). The experiment was not prolonged to the point of observing the fifth stage.

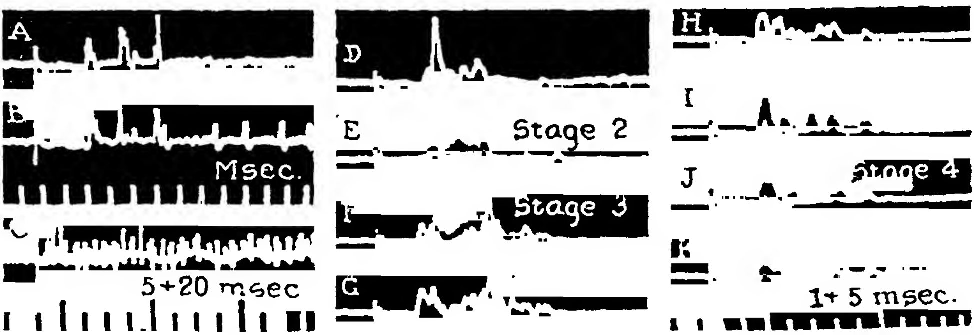

FIG. 5. Influence of eserine on secondary centripetal discharges (A, B, C). D to K—the secondary centripetal discharges reflect the stages of neuromuscular transmission. The time lines refer to the record or records immediately preceding.

In addition to the slow changes in intensity of secondary centripetal activity, Fig. 5 illustrates a fact which holds equally for secondary centripetal discharges in dorsal roots, stimulated and unstimulated ventral roots. The secondary centripetal discharges follow exactly the stimulus frequency at frequencies yielding mechanical fusion of muscle contraction. Granting that some aspect of muscle action is responsible for the initiation of secondary centripetal discharges, the last fact would tend to exclude muscle contraction as the stimulating agent. It is worth noting, since stimulation by muscle action currents is implied as the mechanism of secondary centripetal discharge in an earlier section, that the muscle action potentials remain distinct during a tetanus and furthermore that they undergo characteristic amplitude variations during the several stages of neuromuscular transmission (14).

The locus of stimulation of recipient nerve fibers. The observations on latency (Fig. 3 and Table 1) indicate that the recipient nerve fibers are stimulated at or near their terminations and not at random along some part of their course. In order to substantiate this view, control experiments to demonstrate that active muscles do not stimulate the *intact* nerve trunks (*i.e.* the preterminal portions of the nerve fibers) were fashioned as follows. In addition to the usual root preparation, a small window was made over the hamstring nerves which were located and severed. Otherwise the normal anatomical relationships within the leg were preserved. On stimulating a

IMPEDANCE CHANGES IN FROG'S MUSCLE ASSOCIATED WITH ELECTROTONIC AND "ENDPLATE" POTENTIALS

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INTRODUCTION

IN A previous paper (14) it has been shown that a motor nerve impulse, on its arrival at a blocked myoneural junction, gives rise to a brief subliminal depolarization of the muscle fibre which dies out in the vicinity of the endplate within about 20 msec. This "endplate potential" (e.p.p.) resembles in many of its properties a subthreshold catelectrotonic potential change. In the present paper a further analogy is provided by the diminution of impedance found under both conditions.

The occurrence of a small impedance loss at the *cathode* preceding and possibly initiating the much more drastic propagated change (4, 11), bears an interesting relation to the problem of excitation, the setting up of local responses and their transition into "all-or-nothing" impulses.

The impedance loss during the e.p.p. differs in its initial time course from that accompanying a similar catelectrotonic potential; a comparison of the two effects suggests that, at the neuromuscular junction, a nerve volley may produce an initial membrane change which rises and falls more rapidly than the e.p.p., and this is discussed in relation to the transmitter problem.

METHODS

General technique of impedance measurement

Changes of electric potential and of transverse impedance were measured on the frog's sartorius which was isolated together with its nerve. For impedance measurements, platinized platinum electrodes of 1 or 0.5 mm. width were applied to the muscle transversely. Muscle and electrodes formed one arm of a Wheatstone bridge and were balanced by a combination of variable resistance and capacity in parallel.

A resistance-capacity tuned oscillator as described by Terman *et al.* (23) was used providing sine waves of very low harmonic content. The bridge output was connected to a resistance-capacity coupled amplifier and cathode ray oscillograph. Impedance changes of less than 0.01 per cent could be measured. The response of the amplifier to an instantaneous alteration of bridge balance was complete within less than 0.3 msec.

The 2 Pt wires were perpendicular to the muscle, but because chance asymmetries were found to act as a differential lead for action potentials, recording a potential of reduced size (10–15 per cent) and quickened time course whenever a spike or e.p.p. was set up (7). To avoid its interference with the recorded bridge output, a high-pass filter cutting off frequencies below 2000 c.p.s. was inserted between bridge and amplifier.

The bridge input consisted of alternating current (A.C.) at 5000 c.p.s., its strength being well below half-threshold, probably no more than 10 per cent of the direct muscle threshold. Since this work is concerned only with the time course of the impedance changes, observations at several frequencies were not required. 5000 c.p.s. was a frequency high enough to map out the time course of events during spike and e.p.p., yet low enough for adequate recording of membrane changes (5, 16). The A.C. strength was so low that it did not add appreciably to the excitatory effect of the e.p.p., even if the latter was only slightly

below threshold. Furthermore, halving or doubling the strength of the A.C. merely changed the bridge sensitivity without altering the impedance changes. Thus, the measuring current did not seem to affect the condition of the tissue.

In the following, impedance $Z = R/\sqrt{1+R^2\omega^2C^2}$, phase tangent $\tan\phi = R\omega C$, R and C *being the adjustable parallel resistance and capacity respectively, and* $\omega = 2\pi \times$*frequency* ($=31{,}000$/sec.). $R^2\omega^2C^2$ was always less than 0.2, R about 2000 to 3000 Ω.

During spike, catelectrotonic potential, and e.p.p. a transient diminution of impedance and phase tangent occurs, but while the changes during the spike are of the order of 10 per cent, those during the e.p.p. are only about 0.1 per cent. The most accurate way of tracing their time course would be to unbalance impedance and phase angle at rest and find, by trial and error, such values of R and C as would give balance at various moments *during the change.* However, *as* Cole *and* Curtis (7) *found, the result of this method does* not differ appreciably from the time course of the bridge record itself, obtained with balance at rest or during the maximum change (which implies that the change of complex impedance, in a resistance *vs.* reactance diagram (5), follows approximately along a straight line). Now, with changes of only 0.1 per cent, the method of balancing at various moments becomes impracticable, partly because of residual harmonic content and partly because of slow drift of the resting impedance. For the same reasons, it was inconvenient, and often impossible, to work with sharp balance at rest or at the peak. The procedure usually adopted was to set the bridge slightly off balance and to record the amplitude changes on the oscillograph. A band of oscillations was recorded (*e.g.* Fig. 9) which during the e.p.p. increased or decreased in width, depending upon whether the impedance of the variable bridge arm had been made greater or smaller than that of the resting muscle. In this way, the time course of the impedance change could be obtained with sufficient accuracy, provided that the direction of the complex impedance unbalance, at rest, was in phase with that of the impedance change (*i.e.* coinciding with the direction of the mentioned straight line in the resistance-reactance diagram). This direction was found by adjusting the variable impedance and phase angle so as to get (i) a maximum change of band width; (ii) an opposite, but symmetrical change of band width when the sense of the resting unbalance is reversed; (iii) a minimum phase shift of unbalanced fundamental frequency (as indicated by its relation to the unbalanced harmonic content). In most experiments these conditions were obtained by unbalancing the parallel resistance R, while leaving the capacity C at balance.

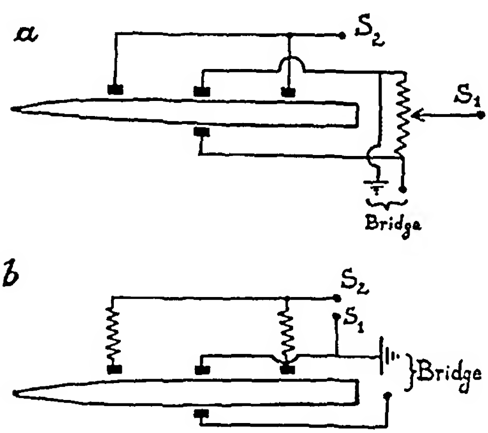


FIG. 1. Sartorious muscle with electrodes for direct stimulation and impedance measurements. *a* and *b*: 2 alternative circuits (see text). S_1, S_2: Stimulating electrodes.

The measurement of the records provided no difficulty in spite of the high amplification and consequent unsteadiness of the baseline (overall voltage amplification of bridge output was about 10^6). A more serious distortion was due to the cathode ray tube, the vertical sensitivity of which changed somewhat at different positions of the horizontal sweep; but this could easily be corrected for.

E.p.p.'s and muscle spikes, due to nerve stimulation, were recorded by using the im-

pedance electrodes connected together as the "active" lead and a Pt electrode on the end of the muscle as reference lead.

Impedance measurements during direct stimulation

As direct stimulus a double condenser discharge was used rising to a peak in 0.8 msec. and falling to one-half in about 2 msec. This particular type of stimulus was chosen because it would set up an electrotonic potential similar in shape to the endplate potential.

The muscle was deeply curarized (curarine concentration of 9 μmol. per l.) so that any changes, *e.g.* endplate potentials, due to excitation of intramuscular nerves would be quite negligible. The stimulus was delivered, and synchronized with the time base, by a Lucas pendulum. The current was led into the muscle in two different ways (Fig. 1), entering either at one or both impedance electrodes and leaving at two other Pt electrodes about 1 cm. away on either side. By using both impedance electrodes as a common stimulating lead, connected together by a high resistance (Fig. 1*a*), potential changes due to stimulus artifact could be minimized. On the other hand, with the arrangement of Fig. 1*b*, it was possible to earth the stimulating apparatus and so to avoid baseline disturbance during the fall of the pendulum. The results were the same with either method. To make the time course of the stimulus independent of electrode polarization, a resistance of at least 50,000 Ω was placed in series with the muscle. Oscillograph records showed that the shape of the current pulse was unaltered when preparation and electrodes were short-circuited.

If the muscle was replaced by a saline soaked wick, no detectable impedance change (*i.e.* less than 0.001 per cent) was produced by currents somewhat stronger than employed in the experiments.

Results

A. *Impedance Changes Elicited by Subthreshold Electric Currents*

At the outset of this work, there were reasons to believe that a subthreshold electric current would not alter the muscle impedance at all. Cole and Curtis (7) had been unable to find an impedance change during the subthreshold "foot" which precedes the propagated action potential, and their work suggested that a change of membrane permeability, and an associated impedance change, would occur only after attainment of a critical depolarization. The present experiments, however, in agreement with Dubuisson (11) and Cole and Baker (4) show that this view must be modified. As illustrated in Fig. 2, any subthreshold current pulse produces an increase of muscle impedance at the anode, and a somewhat greater decrease at the cathode.

The size of the subthreshold impedance changes is relatively small, *e.g.* at threshold for the most excitable fibres the cathodal impedance loss amounts to about 0.5 per cent, which compares with a diminution of about 10–15 per cent during a maximal, fairly synchronous, muscle spike (Section II A).

The time courses of the impedance change is shown in Fig. 3. Its relation to the applied current pulse is of particular interest. The impedance change rises gradually and reaches a maximum during the declining phase of the stimulus. It then falls slowly, in an approximately exponential fashion, decaying to one-half in about 7–8 msec. This time relationship is similar to that of the electrotonic potential of muscle (Section I B), cf. also Schaefer, Schölmerich and Haass (22), and the rate of decay is about the same as that of the e.p.p. (14).

In normal muscle, the time course was approximately the same for all anodal and subthreshold cathodal stimuli, though often the decay was found to be slightly quicker at the cathode than at the anode. If threshold was exceeded, propagated impulses occurred, accompanied by a quick transient impedance loss and followed, after several milliseconds, by a change associated with the contraction (see below). It is interesting to note that, at

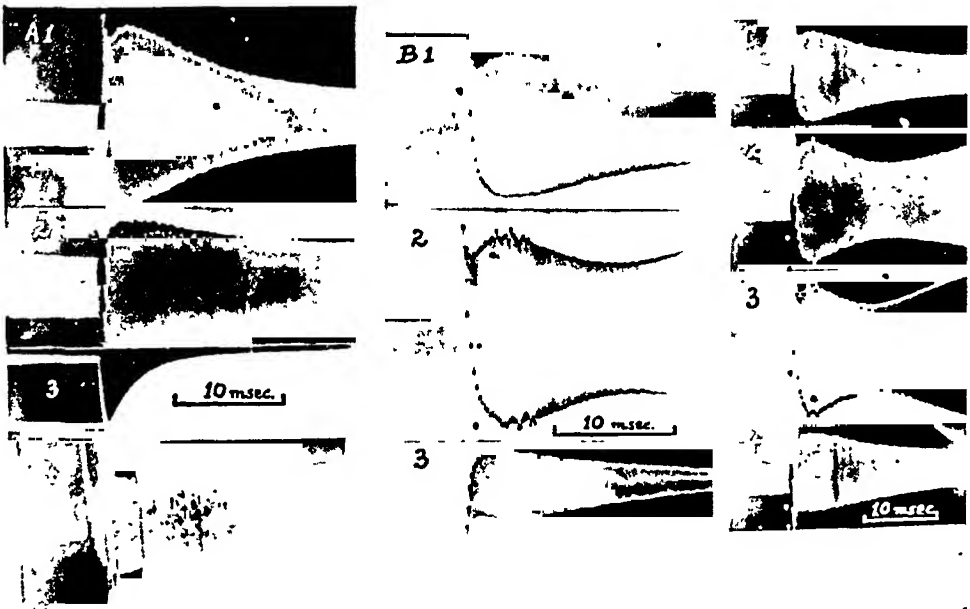


FIG. 2. Impedance changes during direct stimulation. Bridge unbalance, at rest, was of opposite sense at anode and cathode so as to give always an increase of band width. Circuit of Fig. 1*b* was used (hence stimulus artefact not balanced). *A:* 24°C. *1:* cathode, 14 V., impedance drop 0.4 per cent; *2:* anode, 14 V., impedance rise 0.16 per cent; *3:* record of stimulus; *4:* cathode, superthreshold stimulus (lower amplification), note spikes and later change due to contraction. *B:* 22.5°C. *1:* cathode, 33 V., imp. drop 0.29 per cent; *2:* cathode, 38 V., imp. drop 0.41 per cent, spikes and contraction change; *3:* anode, 33 V., imp. rise 0.16 per cent. *C:* 24°C. *1–3:* cathode; *1:* 12.5 V., imp. drop. 0.255 per cent; *2:* 17 V., threshold current, note little spike on top of imp. change (−0.385 per cent) and later contraction change; *3:* 19 V. additional spike and contraction change. *4:* anode, 17 V., imp. rise 0.205 per cent.

threshold, the propagated disturbance takes off at the peak of the catelectrotonic impedance loss. With still greater current intensities the latency of the propagated change diminishes and its size increases, more fibres being involved. This again confirms the general similarity of potential and impedance change (22).

The impedance change during contraction has been studied in detail by Bozler (2) and Dubuisson (12) who have shown that, unless the muscle is fixed rigidly, the observed change is largely due to movement and local thickening. In the present work, no special attention was paid to the contraction change except that it served as a sensitive subsidiary

threshold index. It was absent with subliminal direct stimulation, or with nerve stimulation in completely curarized muscle (Section IIB). It became appreciable only after the spike and its accompanying impedance change had declined to a small remainder. The separation between the impedance changes, accompanying spike and contraction respectively, was largest when recording from the cathode or, in the case of nerve stimulation, from the junctional region, that is before any conduction along the muscle has occurred.

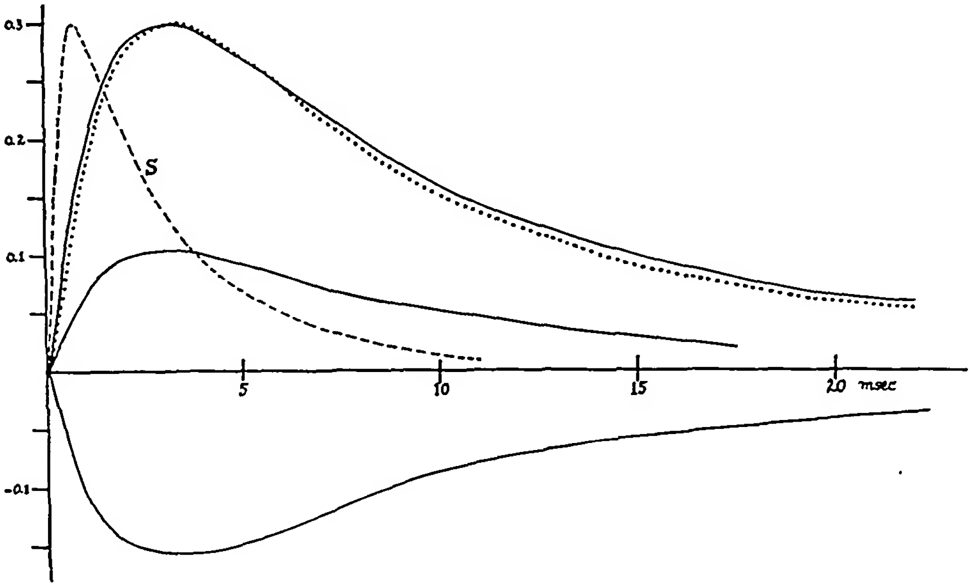
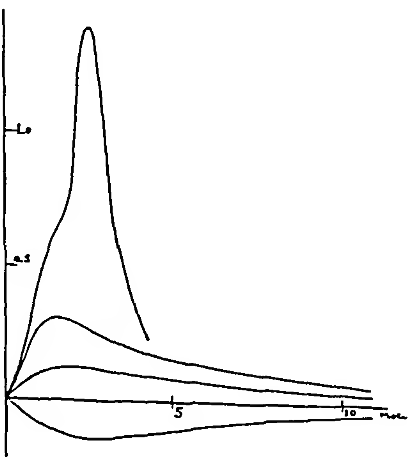


FIG. 3. Time course of impedance changes (full lines) and stimulus S (broken line). 24°C. Ordinates: impedance decrease (upwards) and increase (downwards) in p.c. Current strength, in p.c. of threshold for most excitable fibres, successively from above: 0.78, 0.38; −1.0 (anodal). The *dotted curve* shows the approximate time course of the electrotonic potential (see Section IB).

If the muscle was damaged, *e.g.* by application of a prolonged polarizing current, the threshold increased progressively, while the subliminal impedance changes diminished in

FIG. 4. Impedance changes in injured muscle (see text). Ordinates: per cent impedance loss. Cathodal changes upwards, anodal downwards. Current strength in relative units, successively from above: 1.0 (subthreshold), note spike, 0.73 (below threshold), 0.52; −1.0 (anodal).



size and quickened in their time course. At the cathode, with currents of slightly less than threshold strength, a relatively large quick component of the impedance loss was observed (Fig. 4), possibly the counterpart of a non-propagated response. This effect, though only found in deteriorating muscle, is of some interest, since it may be related to the marked local responses (20, 21; unpublished experiments by Kuffler on single muscle fibres) which have been observed at the cathode under similar conditions.

The relation between applied current strength and impedance change was always non-linear. In Fig. 5 the two variables are plotted, and a character-

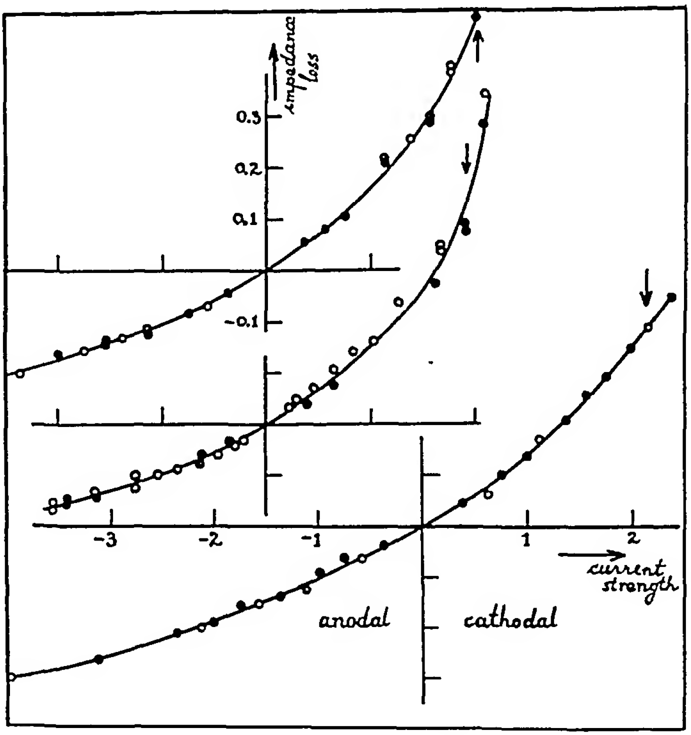


FIG. 5. Relation between impedance change and current strength. Three different experiments. Ordinates as in Fig. 3 (marked at every 0.1 per cent change). Abscissae: Current strength in relative units. (Cathodal currents and impedance loss are represented as positive.) Appearance of spikes marked by arrow. Hollow circles: series of observations with increasing current; full circles: reverse series.

istic curve is shown the slope of which increases continuously at the cathode as the current intensity approaches threshold, while it decreases with increasing anodal current strength. This type of curve may be of considerable importance; however it cannot be interpreted accurately until more is known about the current distribution in the muscle and the absolute values of membrane resistance and capacity.

B. *The Electrotonic Potential of Muscle*

It was desirable to compare the time course of the sub-threshold impedance changes with that of the electrotonic potential. This could not be directly recorded, at the point of stimulation, because the double-condenser pulse introduced a large artifact which could not be balanced without

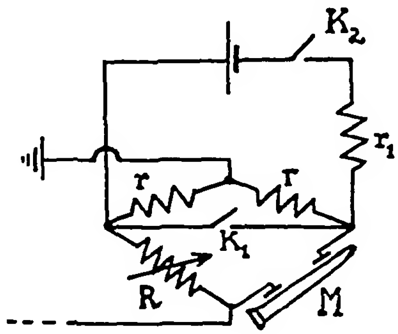


FIG. 6. Circuit for determining time course of polarization potential of muscle. K_1, K_2: break contacts of Lucas pendulum. $r_1 = 0.1$ MΩ. $r = 1000$ Ω. R: balancing resistance. M: sartorius muscle.

distorting the electrotonic potential to an unknown degree. Its approximate time course was then found in the following way. A weak constant current (5–10 per cent of threshold strength, lasting about 60 msec.) was applied to the muscle by means of 2 non-polarizable electrodes (chlorided silver plates in 2 pools of Ringer which were bridged by 10–15 mm. length of muscle; the muscle was connected with the saline pools directly or by 2 bridges of soaked wood, 0.5 mm. thick, so as to make the contact area similar to that in the previous experiments).

The development of the polarization potential at the muscle membranes was recorded as shown in Fig. 6. The preparation was inserted into a direct current bridge, fed by the constant current pulse and balanced at various moments of the current flow, *e.g.* at the beginning or end (Fig. 7). The bridge output gives us then the time course of the back e.m.f. developed by muscle and electrodes. Controls showed that neither electrode polarization nor amplifier distortion were appreciable. (i) If the muscle was replaced by a saline wick, the polarization potential was reduced to less than 2.5 per cent (Fig. 7*c* and *d*). (ii) The amplifier response to a rectangular voltage input reached 90 per cent in about 0.1 msec., and declined to one half in about 0.7 sec. Thus, potentials lasting 60 msec. would be slightly distorted (Fig. 7*a*). This error, however, can be eliminated by adjusting the bridge for balance during the steady state of current flow (Fig. 7*b*).

Thus, the records give the time course of the polarization potential of the muscle itself (*i.e.* of its electrotonic potential). It is approximately exponential, the average "half-time" (*i.e.* time for 50 per cent decay or rise) being about 7 msec. (actually, successive half-times become progressively larger: in the example of Fig. 7, 5.6–7.7–7.9–8 msec.). This is nearly the same as the average time for half-decay of the endplate potential (14).

From these records (Fig. 7) one can determine, by graphical analysis, the approximate time course of the electrotonic potential for any shape of current pulse, *e.g.* for the double-condenser-discharge used above. The analysis is based on the fact that any electric stimulus may be treated approximately as a series of brief constant current pulses, the total electrotonic potential being made up by their summed contributions. The result of such an analysis has been plotted in Fig. 3, for comparison with the impedance changes. Rising period and rate of decay are nearly the same as in the impedance records. It is not possible to say whether there is exact agreement between impedance and potential curves, since the determination of the electrotonic potential is approximate, and the data were obtained from different muscles where a 20–30 per cent variation in time scale was usual. A recent report by Cole (3) indicates that, in the squid giant axon, the impedance change lags somewhat behind the potential. This possibility has not been excluded for the frog's sartorius, but as a first approximation the time courses of subthreshold impedance and potential changes in frog's muscle may be taken as identical.

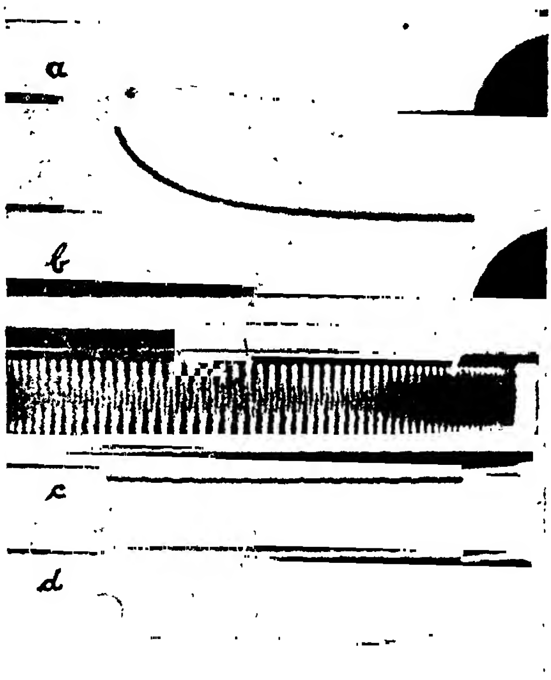


FIG. 7. Polarization potential of muscle during constant current flow. 15°C. *a:* bridge balanced at beginning of current pulse ($R = 9310\ \Omega$); *b:* bridge balanced during steady state ($R = 11410\ \Omega$). *c* and *d:* muscle replaced by saline wick. Bridge slightly unbalanced, in opposite sense at *c* and *d* ($R = 2670$ and $2570\ \Omega$ respectively). The polarization in *c* and *d* is equivalent to a resistance change, during current flow, of less than $40\ \Omega$, *i.e.* 2 per cent of that in *a* and *b*. Time signal 500 c.p.s.

II. Impedance Changes at the Neuro-muscular Junction

The main object of these experiments was to examine further the nature of the local change which a nerve volley sets up in a completely curarized

muscle. Potential and impedance changes were recorded, before and after curarization, at a junctional region of the frog's sartorius (*i.e.* a region of minimum spike latency, or maximum e.p.p.; see 19).

When comparing impedance and potential records, one must take into account that the two changes are recorded effectively at different points, the former being led off from the whole region between the transverse *Pt*-electrodes (see Methods), the latter only from the edge of the *Pt* electrode which faces the reference lead. However, owing to the scatter of individual endplates, the junctional regions of most muscles extend over an area larger than that of the *Pt* electrodes. Within these regions, movement of the electrodes causes no appreciable change in latency or shape of the potential records and so it is safe to compare them directly with the impedance changes recorded from the centre of a junctional region.

A. *Impedance Changes During the Muscle Spike*

In a non-curarized nerve-sartorius preparation, muscle spike and associated impedance change, due to a maximum nerve volley, was recorded

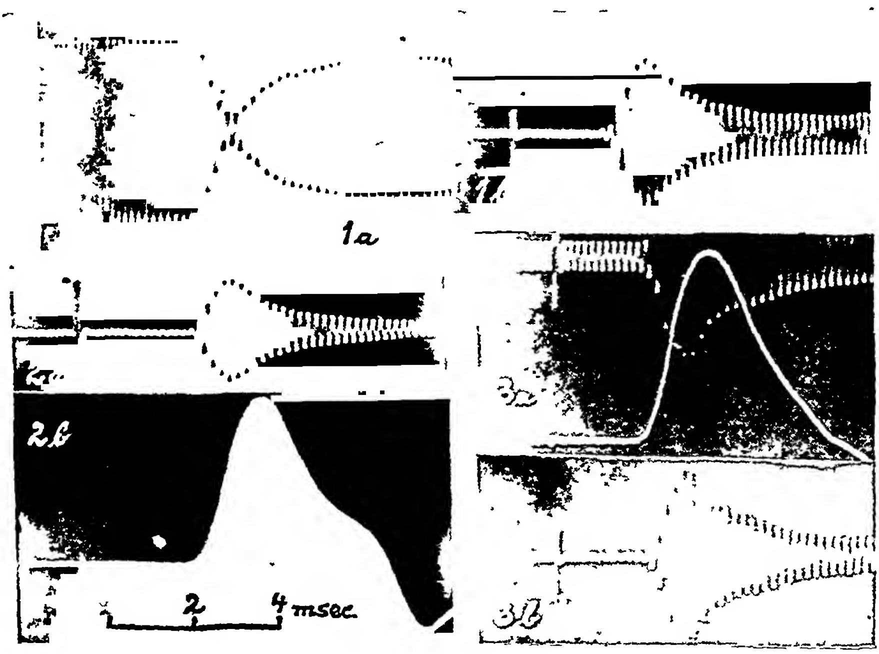


FIG. 8. Action potentials and impedance changes recorded at junctional region of normal sartorius. Maximal nerve stimulus. 1*a* and *b*: 21°C. 8 per cent. Impedance drop. 2 different adjustments of bridge. 2*a* and *b*: 16°C. 6.3 per cent impedance drop; spike 59 mV. 3*a* and *b*: 21°C. 10 per cent impedance drop; spike 63 mV. Note: the last 2 msec. of the impedance record are affected by the beginning of a later change due to the contraction.

from a junctional region (Fig. 8). There was a diminution of impedance and phase tangent of about 10 per cent (mean of 8 experiments: 9 per cent, varying between 5 and 15 per cent). The records are similar to those obtained by Cole and Curtis (6, 7) on Nitella and single nerve fibres, and by Auger

and Fessard (1) on the electric organ, except that the spike effect is followed, after an interval of several milliseconds, by a second impedance change accompanying the contraction (see Section I; cf. 10, 12). Potential and initial

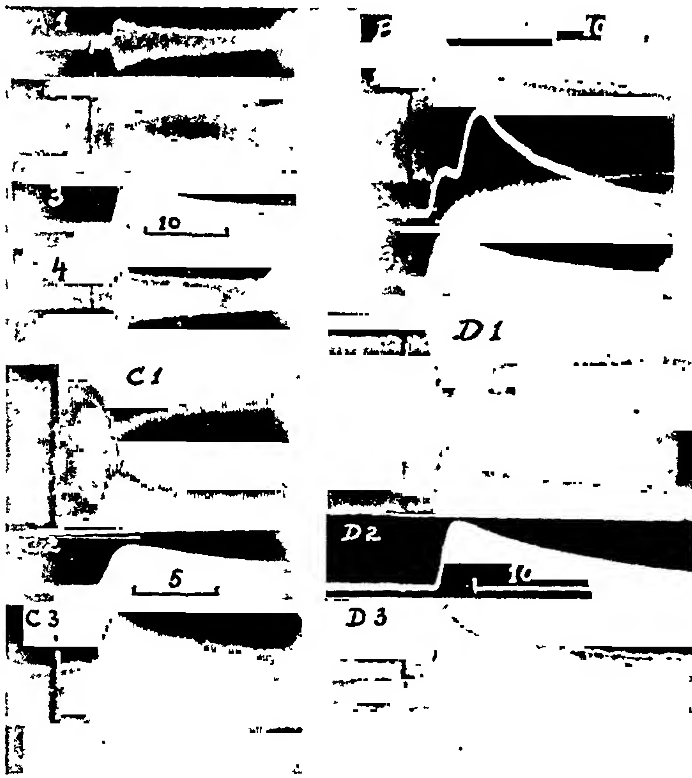


FIG. 9. E.p.p.'s and impedance changes in fully curarized muscle, both recorded from junctional region. Ringer contains five-fold excess of calcium and about 4 μmol. curarine. *A*: 17°C. 0.08 per cent impedance drop (3 different bridge adjustments); e.p.p. 2.7 mV. *B*. 21°C. 0.15 per cent impedance drop, during single e.p.p. (3.4 mV). (Note appearance of small propagating spike on top of second e.p.p.; threshold height of e.p.p. about 7.5 mV). *C*. Faster recording speed. Impedance drop 0.135 per cent; e.p.p. 3.1 mV. *D*. 18°C. Impedance drop 0.125 per cent; e.p.p. 4 mV. Time marks: for *A*, *B*, *D*, 10 msec.; for *C*, 5 msec.

impedance changes begin at about the same time, but the impedance change reaches its peak more quickly, the rising phases, at 22°C., being 1.0 msec. (0.8–1.23 msec.) and 1.4 msec. (1–1.8 msec.) for impedance loss and spike potential respectively.

B. *Impedance Changes in Completely Curarized Muscle*

When neuro-muscular transmission was completely blocked by curarine, a nerve volley invariably gave rise to a small localized impedance loss at the

junctional region, accompanying the e.p.p. (Fig. 9). The amplitude of this change varied with the size of the e.p.p. and thus depended upon the degree of curarization. Furthermore, a second nerve volley following several milliseconds after the first produced a larger impedance change, corresponding with a larger e.p.p. (14). For an average e.p.p. of 2 mV the local impedance change amounted, on the average, to 0.06 per cent. This compares with an impedance loss of 9 per cent during the spike (of 50–60 mV). Thus the impeddance change was reduced by curarization about 5 times more than the potential change.

In order to obtain large e.p.p.'s and so improved accuracy of measurement, the muscle was soaked in Ringer's solution containing about 5 times the normal amount of calcium. With a given concentration of curarine, calcium increased the e.p.p. and at the same time raised the threshold potential required for the initiation of muscle spikes. Consequently, larger subliminal e.p.p.'s and impedance changes were recorded without any interference by spikes or contraction.

The time course of the impedance change showed a very consistent relation to that of the e.p.p. (Fig. 9–11). During the later parts of their decay both curves subsided at nearly the same relative rate; however the initial time courses differed, the impedance change rising and falling more rapidly than the e.p.p. In 14 experiments, at 16–28°C. (mean 22°C.), the rising period of the e.p.p. was 1.9 msec. (1.6–2.7 msec.), while that of the impedance change was only 1.05 msec. (0.85–1.45 msec.).

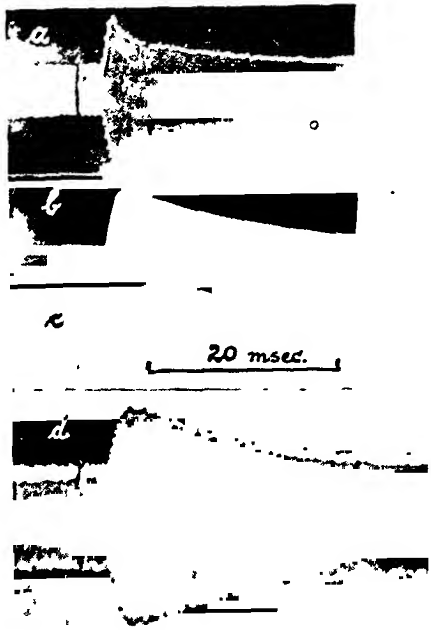


FIG. 10. Effect of eserine in curarized muscle. Calcium—Ringer. *a* and *b*: 4 μmol. curarine. e.p.p., 4 mV; impedance drop, 0.125 per cent. *c* and *d*: eserine 10^{-5} (soaked for more than 1 hour), +8 μmol. curarine (so as to reduce size of e.p.p. below spike-threshold). e.p.p., 3 mV; impedance drop, 0.08 per cent.

After application of eserine (10^{-5}), the rising periods of the e.p.p. and accompanying impedance change are lengthened two- to three-fold (Fig. 10; cf. 13).

The impedance change during a just-subthreshold e.p.p. was of the order of 0.1 per cent, though occasionally reaching 0.2 per cent (and with high calcium Ringer even 0.4 per cent). The change during a subthreshold catelectrotonic potential was considerably larger (about 0.5 per cent Section IA). This however, is only to be expected, since the myoneural junctions and, therefore, the individual e.p.p.'s, are scattered usually over a few millimetres of muscle, while the cathodal current is concentrated at the electrode, where the impedance change is measured.

It is clear from Cole and Curtis' (7) work that the actual change of membrane con-

ductance in a squid axon is much greater than the observed overall change of impedance, which is small because of the shunting effect of inert membrane components and surrounding fluid. For example, Cole and Curtis (7) estimate that, during the spike, the membrane conductance increases 40-fold (the overall impedance loss being only a few p.c.). If this estimate can be applied to muscle it would indicate that the impedance loss during a just subthreshold e.p.p., though only 1/100 of the spike change, is due to nearly 50 per cent increase of membrane conductance.

Discussion

A. *Impedance and Permeability Changes Produced by Subthreshold Currents*

As shown by the above experiments, a subthreshold current passing through an irritable membrane produces not only the well-known alterations of membrane potential (17), but in addition a local impedance change of approximately the same time course. Although no analysis at different frequencies was undertaken, it was clear that resistance and reactance changes at the cathode and during the e.p.p. had the same direction as the much larger changes accompanying the spike. Presumably they are due to the same kind of membrane alteration, which is, according to Cole and Curtis (7), an increase of its ion permeability. The question arises whether the subthreshold potential and impedance changes bear any intimate relation to the process of "excitation," that is to the discharge of the propagating potential and impedance changes.

The experiments (Fig. 5) suggest that the ion permeability of the membrane is a continuous function of the electric potential across it: the reduction of the resting potential at the cathode being accompanied by increased permeability (observed as an impedance loss), and vice versa the increase of potential at the anode by diminished permeability. According to the classical membrane hypothesis, the increased "leakiness" at the cathode would be followed by a further diminution of the resting potential; this, in turn, would cause a further increase of permeability and so on. It seems possible that in this way a progressive depolarization (and a progressive resistance breakdown) may be brought about at the cathode, such as observed during the initiation of the action potential.

After the conclusion of the present experiments, the writer became aware of the recent work by Cole and his coworkers (4, 8) in which essentially the same impedance changes were described in the isolated giant axon of the squid. The changes, at anode and cathode, were measured at various frequencies and interpreted as an alteration of membrane resistance analogous to that accompanying the spike. Moreover, the associated changes in membrane potential were recorded between inside and outside of the fibre, and their time course was found to be similar to that of the impedance changes.

B. *The Impedance Loss Associated with the e.p.p.*

The impedance loss accompanying the e.p.p. persists as long, and in its later phase declines as slowly, as the e.p.p. itself (Section II). The time scale

of this phase of decay is identical with that of the impedance and potential changes following a brief subthreshold current pulse (Section I). These observations reveal a further analogy between e.p.p. and catelectrotonic potential, and they agree with the view (14) that the descending phase of the e.p.p. is largely due to passive dissipation of a subthreshold potential change of the muscle membrane.

The difference between catelectrotonus and this part of the e.p.p. would seem to be merely that the one is produced by an artificial stimulus, the other "naturally," by a nerve volley. It is satisfactory to find that the increase of membrane permeability at the cathode can be reproduced by a motor impulse at a blocked junction, and so cannot possibly be attributed to an artifact or injurious effect of the applied stimulating current.

It remains to be asked why the initial time course of the impedance change differs consistently from that of the e.p.p. This difference, *viz.* a more rapid rise and initial fall of the impedance record, was observed in all experiments, independent of the degree of curarization (cf. Fig. 9, 10). Now it is clear from previous work (14) that, owing to the scattered distribution of individual junctions the time course of the e.p.p. recorded from whole muscle will be somewhat slower than that at the individual endplate. It might be that, for some reason, the overall record of the impedance loss is slowed relatively less than that of the e.p.p. This could be decided only if the experiment can be repeated on a single junction preparation such as described recently by Kuffler (19).

There is, however, another possibility which seems worth considering because it may provide a hint as to the mechanism by which the e.p.p. is produced. The impedance record may be due to two components: (i) one which accompanies the electrotonic change and runs the same time course as the e.p.p. (cf. above); (ii) a rapid initial component which occurs during the building up process of the e.p.p. and is superimposed on the more prolonged electrotonic change. A tentative analysis of the records on this assumption is shown in Fig. 11.

It has been pointed out (14) that the e.p.p., in completely curarized muscle, is built up by a brief transmitter action which lasts little longer than the rising phase of the e.p.p.

The relation between "transmitter action" and e.p.p. is quite similar to that between "fundamental process" and development of tension in muscle activity (15). In the present case, the time factor which causes the potential change to lag behind the "transmitter action" is due presumably to the presence of the membrane capacity.

During this initial "action period" the endplate region of the muscle is being depolarized, either by subliminal action currents from the motor nerve, or by a chemical transmitter substance (acetylcholine) which is released and removed within a few milliseconds. It is interesting to note (Fig. 11) that the "action period" described by Eccles, Katz and Kuffler (14) coincides with the rapid initial phase of impedance loss. Furthermore, both are lengthened appreciably by eserine (Fig. 10).

If the e.p.p. is due to a subthreshold discharge of the muscle membrane by nerve action currents, it would be perfectly analogous to a catelectrotonic potential set up by an applied current pulse. There is, however, no evidence for a rapid component of impedance loss concurrent with the period of extrinsic current flow; on the contrary, the indications are that the impedance change during subthreshold electric stimulation develops at the same rate

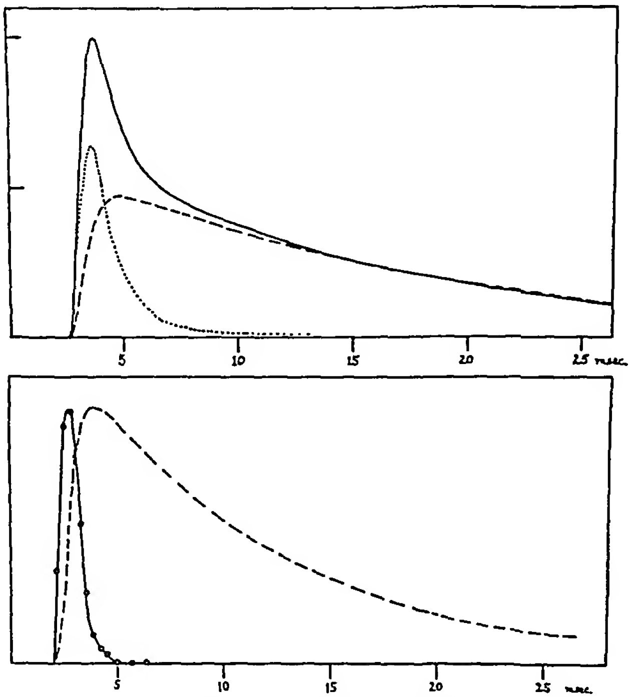


FIG. 11. *Upper part.* Full line: impedance change. Broken line: e.p.p. scaled so as to match falling part of impedance curve. The initial component of the impedance change, which diverges from the time course of the e.p.p., is shown by the dotted line. *Lower part.* Analysis by Eccles, Katz and Kuffler (14*B*, Fig. 19), showing e.p.p. (broken line) and the "probable time course of transmitter action" (full line, hollow circles).

as (cf. Section IB), or even more slowly than, the electrotonic potential (3). One might ask whether the initial impedance change during the e.p.p. is possibly due to the motor impulse and an accompanying membrane breakdown in the nerve terminals, but then one would hardly expect it to be lengthened by eserine and diminished by curarine.

On the other hand, if the depolarization of the endplate region is produced by acetylcholine, a transient diminution of membrane impedance during the "action period" might be expected. On the classical view (24), the presence of a membrane which separates different salt solutions and

restrains selectively the movement of certain ions, is responsible for the resting potential. If we accept this view, then the effect of minute quantities of acetylcholine in reducing the resting potential and making the membrane surface negative (9) would readily be explained, if acetylcholine provides a cation of specifically high penetrating power, or if it is able to render the membrane more permeable to other ions. In either event, release and "action period" of acetylcholine should be accompanied by a diminution of membrane impedance. Thus, the existence of a transient impedance loss during the phase of transmitter action, if established beyond doubt, would bear an important relation to the transmitter problem.

Summary

1. Transverse impedance measurements are made, during direct and nerve stimulation, on the isolated sartorius muscle of the frog.

2. The electric impedance of the muscle is altered at anode and cathode, following the application of a brief subthreshold current pulse. There is an impedance loss at the cathode, and a smaller impedance increase at the anode.

3. These changes decline slowly after the applied current pulse has disappeared. The time course of decay is approximately the same as that of the endplate potential and of the electrotonic potential of muscle.

4. With threshold currents, propagated spikes start at the cathode at a moment when the impedance loss reaches its peak.

5. Impedance changes set up by a nerve volley are recorded at the junctional region of the frog's sartorius. The propagated spike in normal muscle, and the endplate potential (e.p.p.) in completely curarized muscle, are both accompanied by an impedance decrease. Measuring with alternating current of 5000 c.p.s., the overall impedance loss during the spike potential (60 mV) amounts to about 10 per cent, that during the e.p.p. (2–3 mV) to only 0.1 per cent.

6. The impedance change in completely curarized muscle has the same latency and lasts as long as the e.p.p. The rising period of the impedance change is shorter (slightly more than one-half), and its initial fall more rapid than that of the e.p.p.; during the later phase of decay the time course of the impedance change is identical with that of the e.p.p.

7. The prolonged phase of the impedance loss during the e.p.p. is analogous to that produced at the cathode by a direct subthreshold current pulse. The rapid phase of impedance change, which occurs during the building up period of the e.p.p., is discussed in relation to the problem of neuromuscular transmission.

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SOME PHYSIOLOGICAL ASPECTS OF AUDIOGENIC SEIZURES IN RATS*

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SEVERAL investigators have described (11, 17, 8, 1, 6, 12, 19) an experimentally induced, abnormal pattern of behavior in the rat. The behavior is episodic and convulsive-like. It consists of sudden, violent and undirected running, jumping, stiff hopping, and tonic and clonic manifestations; this is followed by an inactive, comatose phase during which there is a lack of response to sensory stimulation, reflexes and righting responses are absent, and the animal may be molded in various positions. The active period of the attack is brief, usually lasting less than one minute; the passive phase may persist several minutes or longer, with gradual return to apparent normality.

The abnormal pattern of behavior and the conditions which evoke it were first described in detail by Maier (11), who likened it to "experimental neurosis." In Maier's original experiments the behavior was initiated by forcing a rat, by means of an air blast, to respond in the Lashley jumping apparatus to a stimulus card which it had been taught previously to avoid. The forcing of a response where available modes of response were restricted or blocked was interpreted by Maier as a "conflict" situation which led to unresolved emotional strain and "neurotic" behavior.

Morgan and Morgan (17) and others (8, 1, 6, 19) have shown that similar patterns of behavior may be evoked in the rat by various types of auditory stimulation, particularly high-pitched sounds, in the absence, presumably, of specific "conflicts." Maier and Glaser (12) produced attacks by auditory stimulation alone, but reported that "conflict" or "barriers to response" enhanced the frequency of, and susceptibility to attacks. Apparently some form of auditory stimulation, usually the hiss of air, was present in all of their experiments.

The present study[1] is concerned only with the type of abnormal behavior induced in rats by high-pitched tonal stimulation. Following the terminology of Morgan and Waldman (18) we refer to the seizure-like behavior induced by auditory stimulation as audiogenic seizures. The purpose of the study was to measure and attempt to evaluate some of physiological components of the reaction. Two physiological variables have been studied, the electro-cortical activity as recorded in the electroencephalogram and heart rate as recorded electrocardiographically.

The physiological changes and the susceptibility of the animals to seiz-

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[1] A preliminary report was made at the annual meeting of the American Psychological Association, September, 1941. (*Psychol. Bull.* 1941, *38:* 579–580).

ures were studied under the following conditions: (i) while the animal was unrestrained, *i.e.*, free to move about in a wire cage; (ii) while restrained, either physically in a special holder, or physiologically by means of partial curarization; and (iii) under conditions of delimitation of the physiological reaction system by bilateral section of the vagus nerves.

METHOD

Only rats found previously to be susceptible to audiogenic seizures were selected for study. Auditory stimulation was provided by a Galton whistle activated by an air compressor under about twenty pounds of pressure. The whistle was set to produce a tone, the major component of which was in the range of 13,000 to 14,000 cycles per sec. The whistle was within 12 to 18 in. of the animal at all times. The testing took place in an electrically shielded, sound-proof room. Two observers were present, one turned on and off the auditory stimulation at intervals, a stimulation period lasting approximately two minutes, the other observer signalled all movements and kept a descriptive protocol of the animal's activities. Electroencephalograms (EEG) and electrocardiograms (EKG) were recorded continuously in an adjacent room.

EEG and EKG were recorded by means of a four channel, Grass inkwriting oscillograph. For recording brain potentials two small, silver disc electrodes were attached by means of collodion, about 1 cm. apart, to the shaved scalp, one over the mid-line frontal, the other over the mid-line occipital region. EKG were recorded from electrodes attached to the two sides of the thoracic cavity. Despite vigorous movements during a seizure little difficulty was encountered in maintaining satisfactory electrode contacts. Light, rubber-covered wires attached to the electrodes did not interfere with the free movement of the animal about the wire cage, which was 12 in. in diameter and 14 in. high.

When animals were restrained physically they were held in a light, adjustable metal holder of cylindrical shape which fits snugly around the body wall. Front and hind legs were held in extension by means of strings attached to the paws. The head was held rigidly in a vice-like rubber lined holder and by means of thread attached to the teeth. In a few cases animals were bound, straight-jacket fashion, with gauze bandage and adhesive tape.

For producing physiological restraint a dilute solution of curare was injected in a leg vein; this caused only partial paralysis and artificial respiration was not necessary. The animals were able to move slightly with difficulty, but not vigorously, until the effects of the curare had worn off. Usually they lay on their stomachs and were unable to stand and walk. Vagotomies were performed under deep ether anesthesia by sectioning the vagus nerves in the cervical region; the animals were then allowed to recover from the anesthesia and were tested for the first time 12 to 24 hr. later.

A brief description of a severe, but essentially typical, audiogenic seizure was presented in the introduction. Although the seizures observed in different animals, and frequently in the same animal at different times, were not always of the same intensity, duration, degree of completeness of pattern, and latency of onset there was however a considerable degree of similarity. In the tabulation of the following results any animal that showed an active phase (violent running, hopping or convulsions) and a passive phase (fixed posture and quiescence or a comatose condition) while free to move about in the cage was said to have had an attack. Similarly for restrained animals any persistent struggling attempt accompanied by a comatose condition was to have been judged equivalent to an attack; as the results show no such attacks occurred.

In addition to the above seizure criteria it was observed that 95 per cent of all animals (both seizure and non-seizure) in this series gave evidence in the majority of non-seizure trials of what we have called "substitute behavior." This consisted of rapid nose or ear rubbing with one or both front paws, teeth chattering, chewing, shivering, shaking, crouching, vibrissae twitching, and restless head or other body movements. One or more of these forms of behavior appeared after the onset of the stimulus and ceased when the tone was turned off.

RESULTS

In attempting to record EEG and EKG during attacks it was thought advisable to reduce the vigorous movements of the animal by physical re-

straint; when this was done no attacks occurred in otherwise susceptible animals. This led to more extensive study of the effect of restraint, both physically and physiologically. Also because of suspected autonomic factors it was decided to test susceptibility of vagotomized animals.

Susceptibility to audiogenic seizures. Table I presents a summary of the results for all animals, showing the susceptibility to audiogenic seizures under the four test conditions. Approximately half of the unrestrained animals had seizures but only 37.5 per cent of their stimulation trials were effective; usually in the non-effective trials some form of "substitute behavior" (as described above) appeared. None of the 14 physically restrained animals had seizures, although 6 of the same animals had seizures when tested in unrestrained trials preceding or following the restrained trials. No overt signs of seizure activity and no characteristic EEG or heart rate changes occurred in 3 partially curarized animals which prior to, and after recovery from, the curare had seizures. Stimulation did not evoke seizures in the two vagotomized animals, although prior to vagotomy seizures were produced in both animals. One of these animals was tested on the second and third days following vagotomy with negative results. Failure to secure seizures in the vagotomized animals was also associated with a lack of heart rate change such as was typical for them during stimulation which induced seizures prior to vagotomy.

Table I. Summary of results of auditory stimulation of animals with a past history of audiogenic seizures when tested under different conditions

Test condition	No. of animals	Total no. of stim. trials	Per cent animals having seizures	Per cent stim. trials resulting in seizures	Per cent successful stim. trials in animals having seizures
Unrestrained	29	182	48.3	18.1	37.5
Restrained	14	41	0.0	0.0	—
Curarized	3	20	0.0	0.0	—
Vagotomized	2	7	0.0	0.0	—

Electroencephalographic results. EEG were recorded in 13 rats during 86 stimulation trials; 8 of the rats had a total of 26 seizures. EEG changes similar to those shown in Fig. 1 and the upper half of Fig. 2, and described below, were observed in each instance that a seizure was induced. The abnormal patterns of activity in the EEG consisted of large slow waves and spike-and-slow wave complexes; these developed just before or simultaneously with the onset of the attack, usually within less than one minute after the onset of the auditory stimulus.

The character of the abnormal activity varied with the phase of the attack; large slow waves or spike-and-slow waves of 2 to 4 per sec. were associated with clonic phases; there were series of spikes or fast low amplitude waves during tonic periods; during comatose periods there was a tendency

toward absence of all electrocortical activity at first, but with a gradual return of the EEG to a normal pre-stimulus level as the animal recovered from coma. The patterns and sequence of changes in the EEG during audiogenic seizures in rats have features in common with the EEG of epileptic patients during attacks and also of non-epileptic patients during seizures

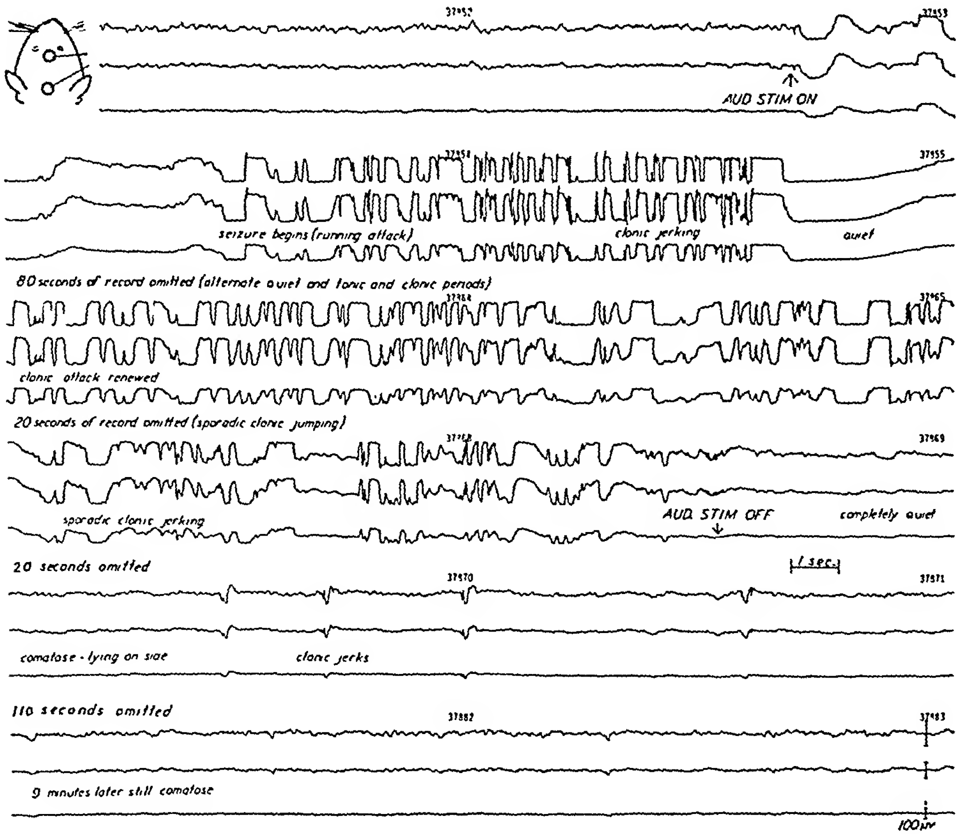

FIG. 1. Electroencephalogram (3 tracings of different amplification) of a rat during an audiogenic attack. (See p. 187).

induced by metrazol or electro-shock. This is especially true of the post-convulsive comatose phase.

Stimulation trials which did not produce seizures, either in restrained (see lower half of Fig. 2) or unrestrained rats, showed no evidence of the marked electro-cortical changes which accompanied seizures, although at the beginning of stimulation there was frequently a 5 to 10-second suppression of normal rhythms which resembles that seen in human subjects when emotionally tense or startled. There was no evidence of "sub-clinical" attacks, *i.e.*, abnormal EEG patterns of convulsive character without overt signs of seizure.

Description of Fig. 1 and 2. Figure 1 shows the EEG (three tracings with different amplification) of an unrestrained rat during a seizure. Normal rhythms prior to stimulation range from 4 to 7 per sec. and are of relatively low magnitude. With onset of stimulation the resting rhythms disappear and irregular slow waves emerge. Eight seconds after the stimulus large slow waves and spike-and-slow wave complexes ranging from 2 to 4 per sec. suddenly appear with the onset of the attack; the attack lasted almost 2 min., terminating with the cessation of the stimulus tone. Just prior to the cessation of the stimulus the

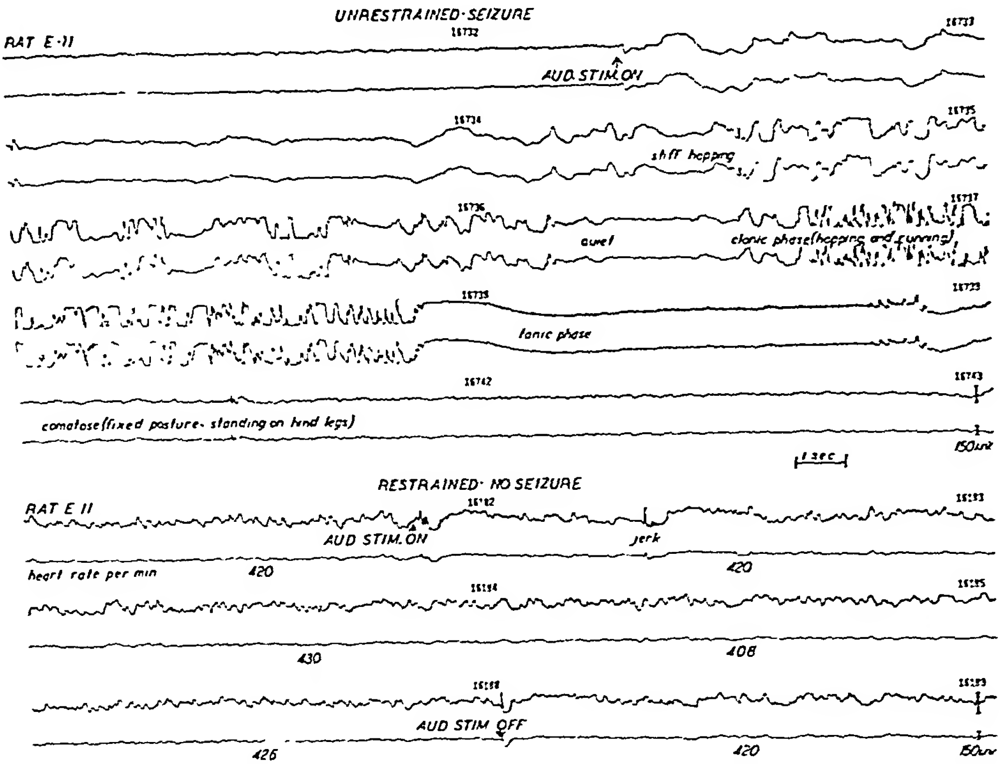

FIG. 2. Electroencephalograms (2 tracings of different amplification) of the same rat during auditory stimulation which evoked an attack (top) when animal was unrestrained, but failed to do so (bottom) when animal was restrained. (See p. 188.)

EEG disturbance abated and the animal sank to the floor of the cage in a comatose condition, remaining thus for more than ten minutes. During the comatose phase electro-cortical activities were at a minimum, pre-stimulus rhythms were barely visible at first, but gradually returned with time. Occasional isolated clonic jerks occurred during this phase; these were associated with single-spike-and-slow wave complexes.

The upper half of Fig. 2 shows the EEG (two tracings with different amplification) of another rat during an unrestrained seizure trial. Irregular slow waves appeared at the onset of stimulation. Twenty seconds later the pattern of activity in the EEG changed markedly and the attack began with stiff hopping behavior; this was followed by a brief quiescent period and then a clonic phase consisting of hopping and running; a tonic phase followed and finally a semicomatose condition during which the animal maintained a fixed posture, standing on hind legs with forelegs against the wire cage. Variations in the pattern of the EEG are associated with tonic, clonic and quiescent periods during the attack.[2] The lower

[2] The EEG are difficult to appraise since some of the electrical activity recorded is due undoubtedly to artifacts introduced by the vigorous movements of the animal. Control

portion of Fig. 2 shows an EEG from the same rat during a restrained stimulation trial which did not produce a seizure. Except for a 5-second period immediately following the onset of the stimulus, during which the normal pre-stimulus rhythms were abolished, no marked abnormalities of the EEG appeared and there was no attack. Inserted heart rate values show that there was no pronounced change in heart rate at onset or cessation of the stimulus.

Electrocardiogram and heart rate changes. Early in the study pronounced changes in heart rate were observed before, during and after the active phase

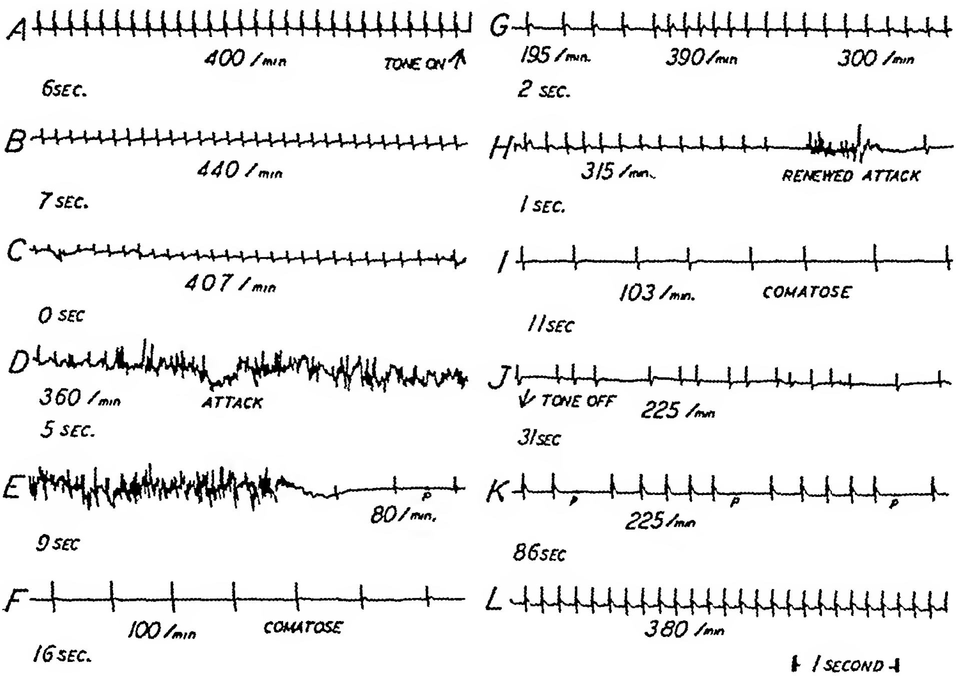

FIG. 3. EKG records before, during and after an audiogenic seizure. Note change in EKG and rise in heart rate after stimulus (B) with subsequent (C and D) decrease just prior to attack (D and E). Profound slowing of HR occurs during comatose phase following attack (F). In (G) HR increases prior to a renewed, abortive attack in (H). HR is slow, and irregular during comatose state in (I, J and K); QRS or ventricular component blocked on some beats in (K), only P-wave is present. In (L) HR returning to pre-stimulus value with recovery from comatose state approximately 3 min. after end of attack. Time intervals between sections of record indicate omitted portions.

of an attack. Whether a seizure occurred or whether only a form of "substitute behavior" (nose or ear rubbing, teeth chattering, etc.) appeared there was almost invariably, following the onset of the stimulus, a striking change in heart rate. In unrestrained animals, especially in those having seizures, three types of heart rate effects have been noted; these in order of their pre-

records with electrodes on other parts of the body have not shown the same kind of patterns, however. Attempts to record seizure patterns in restrained animals have not met with success since attacks were not evoked.

dominance are: (i) a sharp increase of considerable magnitude, with frequently a decrease just prior to an attack, (ii) a sharp decrease, usually followed by an increase prior to an attack, (iii) alternation of acceleration and deceleration, but usually with an over-all increase predominating. The type of heart rate change seemed to bear a consistent relationship to the level of heart rate at onset of stimulation: with initial heart rate at 350 to 400 per min. effect (i) occurred most frequently; when heart rate was initially high

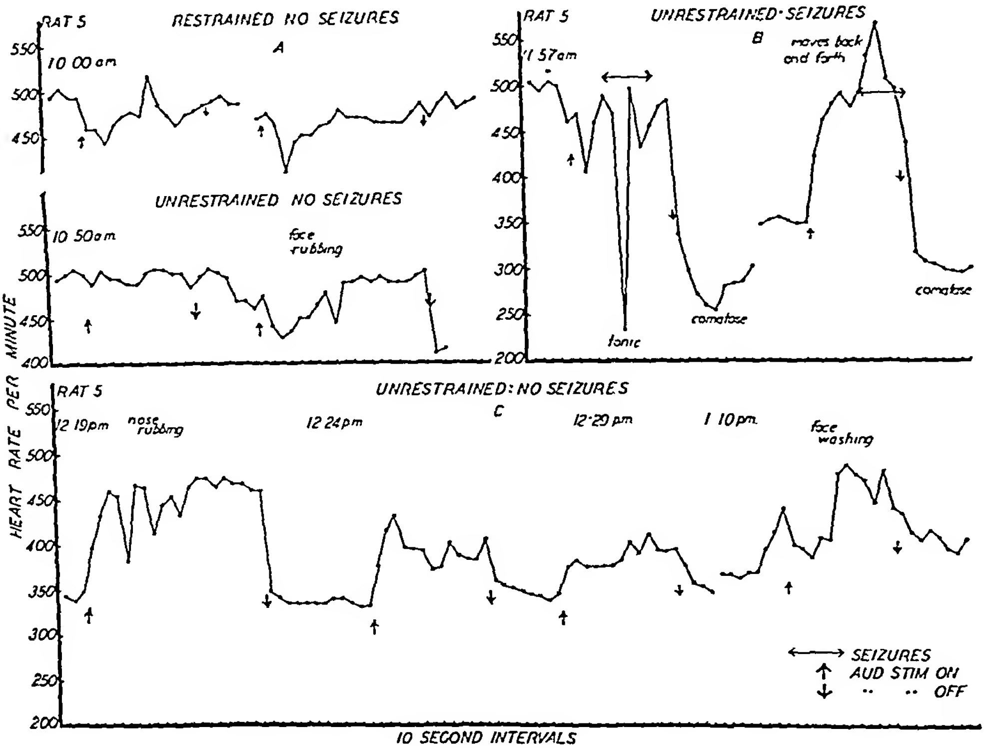

FIG. 4. Heart rate for 10-second intervals plotted for the same rat during restrained and unrestrained stimulation trials. (See p. 192.)

(450 to 500 per min.) effect (ii) occurred. Type (i) effect is illustrated in Fig. 3, and 5A. Type (ii) effect may be seen in Fig. 4B and 5C. With cessation of stimulation a drop in heart rate frequently occurred.

During the active phase of an attack heart rate was usually elevated (in part due to vigorous activity), but with occasional marked, though brief, decelerations. With the onset of the passive or comatose phase heart rate fell sharply to 100 to 300 per min., from which level it recovered gradually. In cases of more profound slowing there was frequently irregularity of the beat and in two instances during the comatose phase frequent omission of the QRS or ventricular component of the EKG (see Fig. 3K) when the

progressively and at the same time become earlier. The refractory period affects the response to the testing nerve impulse less and less. The earlier appearance of the diphasic wave is due mainly to quicker conduction in normal than in refractory muscle. Thus the earliest response to reach the grid lead propagates over the stretch of 1.8 mm. at an average speed of 0.9 per msec., while in normal muscle the rate increases to 2.2 per msec. Such slowed conduction had been already found in refractory nerve (9).

The smallness of the diphasic responses in partly recovered muscle, as compared with the control response (N) indicates that the impulses recorded at the grid have not yet reached their full size. In the experiment of Fig. 2 no attempt was made to investigate whether all the impulses showing a diphasic wave eventually reach their normal size and propagate along the whole muscle fibre after passing the grid lead. From comparison with other experiments it seems likely that the response at 2.8 msec. would sometimes "grow up," while at other times would die out locally (cf. Section C).

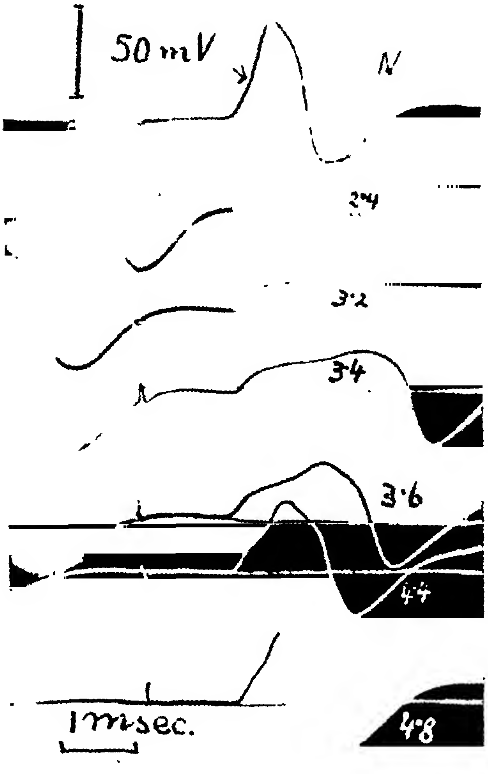

FIG. 4. Action potentials at the neuromuscular junction added by a second nerve impulse (N_2) at the indicated intervals in milliseconds after the first nerve impulse (N_1). The base line is formed by the response to N_1 alone. (At 3.4 msec. interval a base line shift has occurred.) N_2 is fired at a constant position on sweep. Arrow in top record indicates e.p.p. step in the response to nerve stimulation in normal muscle. At intervals of 3.4 to 4.8 msec. during the refractory period the e.p.p. step is more pronounced (cf. also Fig. 5.)

B. Recovery after nerve stimulation

A muscle impulse was initiated by nerve stimulation (N_1) and at various intervals afterwards the recovery of the muscle fibre was tested by a nerve impulse (N_2). Responses due to N_2 during the partial refractory period of the muscle are shown in Fig. 4 and are plotted in Fig. 5 to allow a better analysis. As before, the timing of the conditioning impulse (N_1) was varied while N_2 was kept at a constant position on the sweep. Zero interval for the MN series gave simultaneous M and N spike peaks (see above), hence, with similar intervals in the MN and N_1N_2 series the recovery is tested at the

same time after the conditioning spike. Moreover these two series were taken in quick succession on the same muscle fibre at a constant temperature. The earth lead was left at the endplate region, but the interelectrode distance was 1.5 mm. for the N_1N_2 series. Accordingly the first phase of the N response in Fig. 4 is the same as that in Fig. 2 while the diphasic wave appears earlier.

After a nerve impulse has been sent down to the muscle fibre, further indirect stimulation is prevented for 1.0–1.5 msec. at 18–20°C. by the refractoriness of the nerve. The earliest second nerve impulse finds the muscle

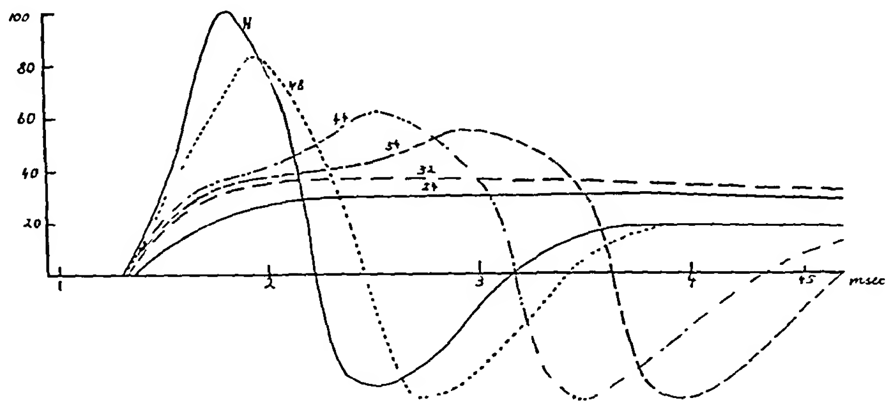

FIG. 5. Experiment of Fig. 4. Action potentials (as percentages of peak potential) added by the second nerve impulse (N_2) to first response (N_1) at intervals indicated in milliseconds (see text).

still refractory so that it cannot give a propagated impulse. Thus at 2.4 and 3.2 msec. interval only an e.p.p. is set up similar to the potentials at *MN* 1.2 and 2.0 msec. intervals of Fig. 2 and 3. A sudden change occurs at 3.4 msec., where besides the e.p.p. a small spike component appears. At the grid lead however a large diphasic wave is recorded, thus showing that at that distance from its origin the impulse has reached approximately normal size. The diphasic wave appears to be even greater than in the control N, but, as shown in a later paper, allowance has to be made for the background e.p.p. The further recovery from the refractory period is revealed at longer intervals. The spike component becomes bigger, appears after a shorter delay, and the diphasic wave is earlier.

In comparing the responses after antidromic (Fig. 2 and 3) with those after nerve stimulation (Fig. 4 and 5) a striking difference becomes evident. A propagated response is set up at a shorter interval with *MN* than with N_1N_2—compare the respective 3.2 msec. responses. Further, these responses at comparable intervals attain a bigger size in the *MN* series. Figures 4 and 5 also show that with the N_1N_2 series there is a much longer delay for initiation of the spike. For example at 3.4 msec. the spike peak is delayed by 1.2

msec., while with the MN series (Fig. 3) the delay is never longer than 0.3–0.4 msec. as compared with the control.

Thus the refractory period at the endplate region following an impulse set up by nerve stimulation is longer than the refractoriness after an 'antidromic' muscle impulse. This has already been found by Eccles and Kuffler (3) in cat's soleus and was attributed to the e.p.p. Eserine which greatly increases and prolongs the e.p.p. at the same time further lengthens the refractory period (2).

A further difference between the MN and N_1N_2 series is that the first propagating response after N_1 'grows up' more quickly and also propagates

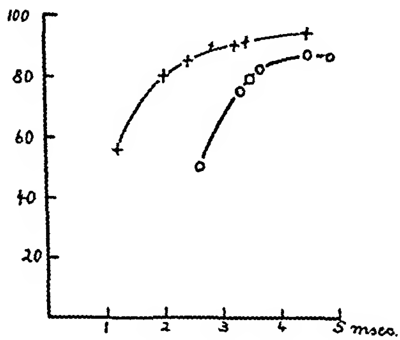

FIG. 6. Experiment shown in Fig. 2 and 4. Ordinates: the rate of rise of the e.p.p.'s, set up during the refractory period in the MN and N_1N_2 series, as percentage of rate of rise in normal muscle. Abscissae: response intervals in msec. Crosses, MN series; circles, N_1N_2 series.

at a faster rate than the first response after the antidromic. Judging from the size of the diphasic wave the 3.4 msec. response in Fig. 4 and 5 has reached its normal height 1.5 mm. from its origin and propagates at an average rate of 1.5 msec. over that stretch as compared with 0.9 per msec. at 2.8 msec. interval in Fig. 2, while the speed for normal muscle is 2.2 per msec.

In Fig. 6 the recovery of the rate of rise of the e.p.p.'s during the refractory period has been plotted for the MN and N_1N_2 series of Fig. 2 and 4. The larger and more prolonged refractoriness in the N_1N_2 series is well seen. At any given interval after the conditioning muscle spike the rate of rise of the e.p.p. is much steeper with the MN than with the N_1N_2 series.

Lengthened latent periods (shock artifact to potential rise) as seen in Fig. 5 for short intervals, *e.g.* at 2.4 msec. are usually observed and are most likely due to slowed propagation of the second nerve impulse in refractory nerve (9).

C. Abortive impulses during the refractory period

In sections A and B it has been pointed out that an impulse initiated during the refractory period at the endplate region gradually gains its full size as it propagates away from its origin. In Fig. 2 at 2.4 msec. interval an impulse was set up at the endplate region but did not propagate along the whole muscle fibre. More information about such "abortive" impulses has been obtained in the following experiments.

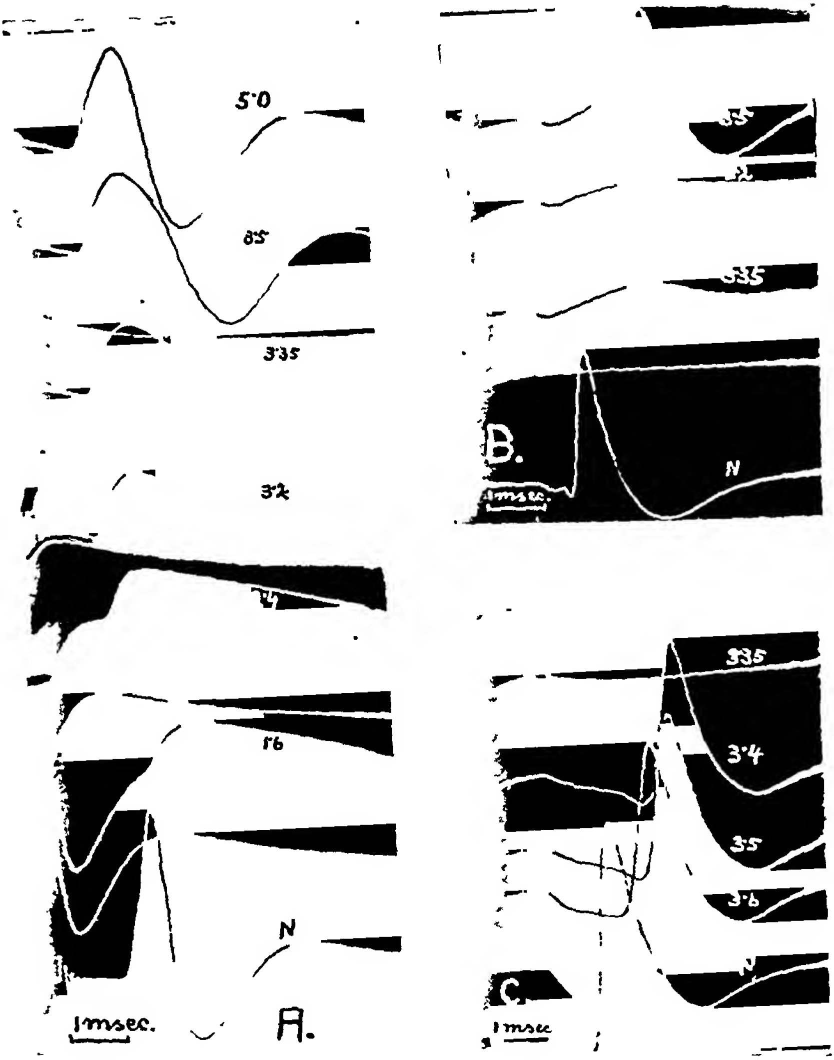

FIG. 7. Recordings in the saline-paraffin interface at different positions on the muscle fibre. Action potentials set up by a second nerve impulse during the relatively refractory period at intervals indicated in milliseconds. A base line is not illustrated in each record. The lowest records of A, B, and C, show the responses to nerve stimulation in normal muscle at different positions on the muscle fibre. A. Action potentials recorded at the endplate, B. at 0.45 mm., C. at 1.0 mm. distance from the endplate.

With interfacial recording the extent of these abortive impulses is better shown, because they produce a diphasic wave immediately they leave the region of the "endplate" electrode. Moreover, this electrode makes a more sharply localized contact with the muscle fibre and the spread of the abortive impulses can be readily determined by shifting the electrode to

various distances from the endplate. The responses stayed constant for such long periods—half an hour or more—that this method gave a reliable indication of the impulse spread.

Figure 7A shows a series of responses in partially refractory muscle with the localized lead at the endplate zone. The same series was recorded immediately afterwards 0.45 and 1.0 mm. away from the endplate and some of the critical intervals are illustrated in Fig. 7B and C. Finally, repetition of the series at the first position showed that no significant change had occurred. In the plotted records of Fig. 8, N_2 adds at 3.5 msec. interval a

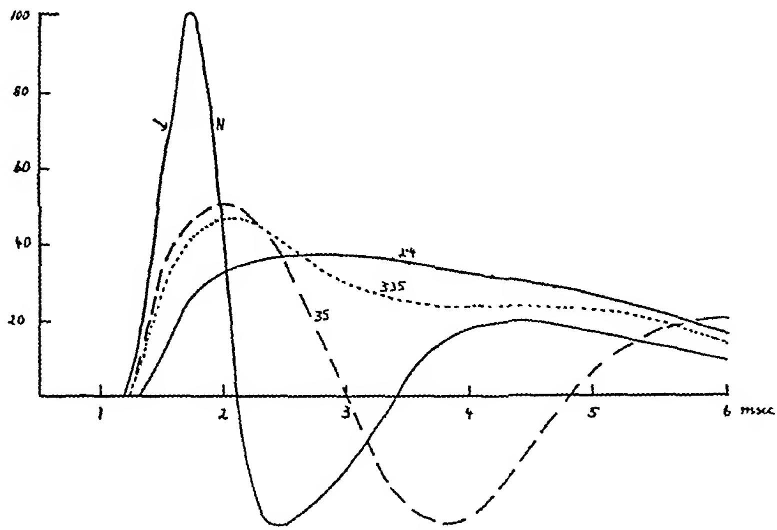

FIG. 8. Some responses of the experiment of Fig. 7A. Potentials as percentages of peak-potential. Arrow indicates e.p.p. component of rising phase.

potential with a full size diphasic response. It has set up a fully propagated impulse. At 3.35 msec. there is a small positive dip in the background e.p.p. if comparison with the 2.4 msec. response is made. The positive dip has a similar time course as the above diphasic wave. This suggests that a small impulse has propagated a short distance from the endplate and then died out. Proof for such an abortive impulse is found in the recording of Fig. 7B, 0.45 mm. away from the endplate, where a small spike is actually observed. The same interval 1.0 mm. from the endplate (Fig. 6C) does not show a spike response at all, only an inversely recorded potential.

This inverse recording is seen also in the initial positivity of propagated spikes of Fig. 6B and C, and is due to the well known effects of current distribution when leading some distance from local potentials in fibres immersed in a large conductive medium (1).

Thus it can be concluded that at 3.35 msec. interval an impulse is set up which propagates as much as 0.45 mm. from its origin and dies out be-

fore it reaches 1.0 mm. For the 3.2 msec. interval the propagation distance has not been determined accurately in this experiment; it is less than 0.45 mm., as comparison with the pure e.p.p. at 2.4 msec. (not illustrated) shows 3.4 msec. is a critical interval at which the impulse sometimes dies out and sometimes propagates (Fig. 7C). The spike peak at this and the longer intervals of Fig. 6C is appreciably later than in the normal muscle. This is due to the slowed conduction in refractory muscle (cf. Sections A and B).

In all the 28 experiments in which double *N* stimulation was used similar findings as in Fig. 7A were obtained. In 8 of these experiments it was shown, by moving the electrode, that a small positive dip following the e.p.p. peak (as in 3.2 and 3.35 msec. Fig. 7A) represents an impulse which propagates decrementally some distance, but never more than 1.0 mm.

Discussion

The present experiments have demonstrated the occurrence of "abortive" impulses during the relatively refractory period. They represent a transitional condition between blocked and normal neuromuscular transmission. Further, in partially refractory muscle the impulse gradually "grows up" as it leaves the endplate region.

The abortive impulses and the "growing up" process are not a special property of the refractory muscle. Subthreshold stimulation of normal nerve gave responses which died out locally, while with just threshold stimulation these local responses grow up to fully propagating impulses (12, 10). Moreover, similar observations have been made recently in normal isolated muscle fibres (unpublished experiments).

In the relatively refractory period the excitability of the muscle fibre is depressed and the e.p.p. has to persist longer to set up a propagated spike (Fig. 2 and 4). Further, the conditions for propagation in partially recovered muscle favor the development of "abortive" impulses and more gradual "growing up." Even under such conditions it is difficult to distinguish between the e.p.p. and the earliest abortive response, unless the latter propagates an appreciable distance. These two processes might be better separated by investigating their impedance changes, as was done by Katz (13) for the e.p.p. alone.

Although local responses can be set up by direct stimulation anywhere along the muscle or nerve fibres, they are greatly modified by the conditions under which they arise at the junctional region. Thus it appears that abortive impulses do not propagate further than 1 mm. if the refractoriness of the muscle is due to a nerve stimulus. Their size is difficult to assess, as they are recorded superimposed on the e.p.p. The total potential height may be as much as 50 per cent of the normal muscle spike potential, but about half of this is due to underlying e.p.p. When the conditioning impulse is set up antidromically responses may die out after having propagated decrementally up to 2 mm.

The eventual propagation of a small impulse is dependent on the ex-

citability gradient along the muscle fibre. Its survival depends on its ability to reach a more fully recovered and therefore more excitable part of the muscle fibre.

The action of the e.p.p. affects the excitability gradient in two ways. As shown in section B and C a cathodal lengthening of refractory period occurs (5) and thus the setting up of an impulse at the endplate region is greatly impeded. The excitability in this case gradually increases with the distance from the endplate, *i.e.* as the catelectrotonic depressant action of the e.p.p. diminishes. This explains the quick "growing up" in the double N series (Fig. 3). The small response reaches more and more excitable muscle as it leaves the endplate region.

On the other hand, an antidromic muscle impulse sets up a shorter refractory period on account of the absence of e.p.p. The second impulse therefore arises relatively early during the refractory period. The excitability gradient in this case will be different from above. The second impulse propagates closely behind the previous one and as it leaves the endplate region rapidly loses the support due to the catelectrotonic spread of the e.p.p. Thus in contrast with the N_1N_2 series a local response has been found to travel much further before reaching a condition at which continued propagation is assured. During the refractoriness following an antidromic impulse abortive impulses may reach up to 35 per cent of the normal spike potential height and they may propagate decrementally as far as 2 mm.

Summary

Electrical responses set up by a nerve impulse at the junctional region during the refractory period have been investigated in the single nerve-muscle fibre preparation of the M. adductor longus of frog (*Hyla aurea*).

1. Three types of responses can be recorded at the junctional region during refractoriness: (a) an endplate potential (e.p.p.); (b) "abortive" impulses (local responses); (c) fully propagated impulses.

2. During the slow propagation of an impulse away from the endplate zone in the refractory muscle the impulse may either 'grow up' to its full size and speed, or die out (abortive impulses). The dying out and the 'growing up' process occurs over 1 or 2 mm., according as the conditioning muscle impulse is set up by a nerve impulse or by direct stimulation. The abortive impulses may attain about 30 per cent of the full spike potential height.

3. The e.p.p. set up by a conditioning nerve impulse lengthens the muscle refractory period.

4. The effect of the e.p.p. in setting up muscle impulses and in modifying their spread from the myoneural region is discussed.

I wish to thank Drs. J. C. Eccles and Bernhard Katz for their valuable help and assistance during the course of these experiments and the National Health and Medical Research Council of Australia for equipping and maintaining the workshop in which most of the apparatus was made.

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EFFECT OF ESERINE ON TRANSMISS

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(Received for publication February 10, 1

INTRODUCTION

IN A SERIES of papers (19, 14, 15, 16, 17, 35, 36, 32) the "endplate potential" (e.p.p.) which a motor nerve impulse myoneural junction have been studied in detail. The relation to the muscle membrane and its role in initiating propagated pulses have been analyzed to a large extent, but the mechanism by which the e.p.p. is produced remains unknown. There are indications that the e.p.p. is set up by a chemical membrane action rather than by extrinsic currents from the motor nerve, but no decisive evidence is available. To obtain further information the effect of eserine on the e.p.p. was studied (18, 14), in particular the question how far its effects can be attributed to the protection of a transmitter substance against enzymic destruction.

The action of eserine on neuromuscular transmission has frequently been studied, but, with the exception of Feng's recent work (22), attention has been focused on the setting up of repetitive muscle responses, on Wedenski inhibition, and on localized contractions, rather than on the changes of membrane potential at the myoneural junction. There is good evidence (14, 15, 36) that the local potentials are an essential link between the transmitting agent and the setting up of muscle impulses or the production of increased or lowered excitability at the junctional region of the muscle fibre. These local potentials will, therefore, give us a more direct indication of intensity and time course of the transmitter action. The question of the repetitive muscle response and of the inhibitory effects of eserine will be dealt with in the second part of this paper, where it will be seen that they are probably entirely due to the changes in local potentials at the endplate region.

METHOD

The experiments were made during 1938–1941 on circulated cat's or isolated frog's nerve-muscle preparations (19, 33) and in a few cases on a single nerve-muscle fibre (35). The potential changes were recorded with a capacity-coupled amplifier, the fall of its response being adjusted as required by varying the size of the coupling condensers (time for one half decay being varied between 0.2 and 3.5 sec.)

Application of drugs. (a) Eserine was given intravenously to cats in doses from 25 μg. to 10 mg. per kg., combined with atropine 100 μg. per kg. The effects reached full in-

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† Dr. Eccles' paper was posted from Australia on December 17, 1941. Owing to conditions prevailing in the South Pacific he has not been able to examine proofs. Drs. Katz and Kuffler, who have papers in this issue, were also unable to read their proofs.

RESULTS

I. THE EFFECT OF ESERINE ON THE LOCAL POTENTIALS AT THE ENDPLATE REGION

A. Non-curarized muscle

1. *Effects of single nerve volleys.* The characteristic effect of eserine is an increase and lengthening of the local negative potential change which is produced at the endplate region by a nerve volley and which persists be-

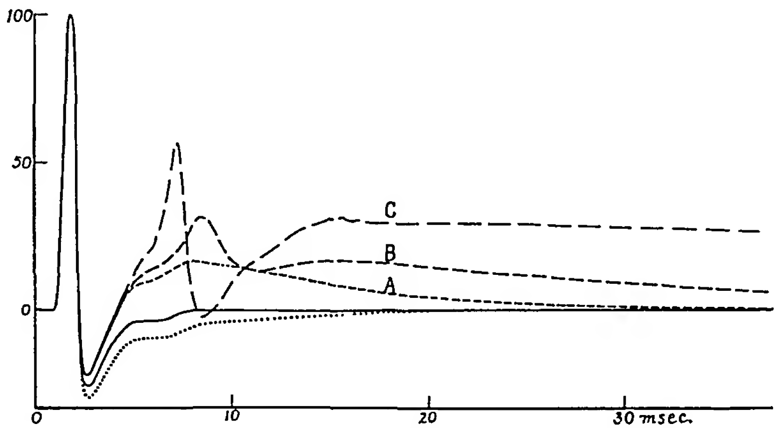

FIG. 1. Cat soleus. Action potentials set up at endplate zone by a single nerve volley. Continuous line, eserine-free muscle. Broken lines show action potentials with progressive eserinization: *A*. 150 μg.; *C*. 600 μg. eserine per kg.; *B*. 40 min. after *C*. Dotted line shows approximate time course of muscle spike potential uncomplicated by endplate potential (17). Simultaneous electrical records from nerve showed no nerve after-discharges except in *C*.

yond the initial muscle spike (17, 35, 36). The initial spike response is not changed, nor has eserine any effect on the muscle action potential set up by direct stimulation. An example is shown in Fig. 1. With progressive increase of the eserine dose, the effect becomes greater and eventually leads to the discharge of repetitive muscle spikes.

It will be shown later that the large effect in Fig. 1*C* was actually due to a cumulative action of repetitive nerve discharges, such as have been

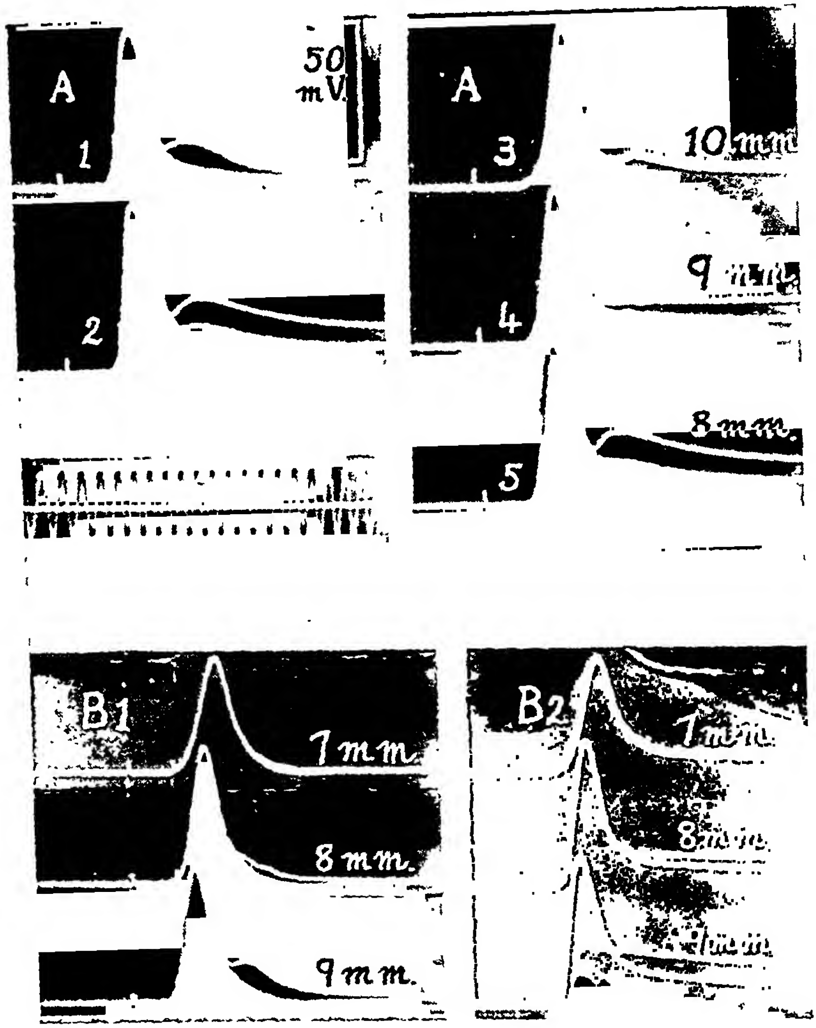

FIG. 2. Frog's sartorius, monophasic recording. Effect of eserine on e.p.p. Superimposed records of potentials at junctional region, set up by single (N_1) and double (N_1N_2) nerve volleys. *A*. 18°C. *1:* normal, N_1, with N_1N_2 at 2.8 msec. interval superimposed; N_2 adds a pure e.p.p. *2:* same, after eserine 10^{-5}. *3–5:* as 2, but N_1N_2 interval 3.2 msec., recorded at 10, 9 and 8 mm. from pelvic end respectively. Note slower rise of e.p.p. as lead is moved away from junctional region (8 mm., cf. timing of spike). *B*. 16°C. N_1N_2 at 2.4 msec. interval; position of earth lead, in mm. from tibial end, shown in figure; endplate focus at 9 mm. *1:* normal; *2:* after eserine 10^{-5}. Time signal: 1000 c.p.s.

found by Masland and Wigton (40) in the eserinized cat. However, with the smaller eserine effects, there were no nerve after-discharges, and the potentials of Fig. 1*A* and *B* were strictly due to a single motor volley.

In the frog, repetitive nerve impulses occur rarely, if ever (Section III; cf. also 23). As a consequence, the marked effect of Fig. 1*C* was not obtained

with single shocks, but could readily be reproduced by repetitive nerve stimulation (Fig. 4).

2. *Effects of double nerve volleys.* In a normal nerve-muscle preparation, a second nerve volley reaching the junction during the refractory period of the muscle sets up a pure endplate potential (e.p.p., Fig. 2; cf. 17, 19). The time course of this e.p.p. does not differ much from that of completely curarized muscle (15), *e.g.* its duration in the frog is 20–30 msec. Eserine, in a concentration of at least 10^{-5}, lengthens this potential ten times or more (Fig. 2). As will be shown later, with a sufficient dose of eserine and a brief burst of repetitive nerve volleys, the endplate negativity can be lengthened to several seconds.

Spatial spread of local potentials. Eccles, Katz and Kuffler reported (15) that the e.p.p. of curarized muscle spreads electrotonically along the muscle

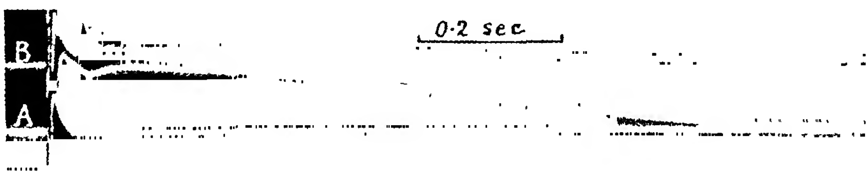

FIG. 3. Cat soleus. Action potentials at endplate zone 600 μg. eserine per kg. *A:* superimposed records set up by single nerve volley and double volley at 2.5 msec. With double volley note prolonged additional potential (the slow wave) rising from the initial e.p.p., It is slightly shortened by amplifier decay ($\frac{1}{2}$ in 2 sec.). *B.* double volley at 51 msec. sets up small prolonged potential.

membrane and that the time course of the spreading potential becomes progressively slower at increasing distance from the junction. Similarly the prolonged eserine potentials rise much more slowly at a point 1–2 mm. away from an endplate focus than at the focus itself (Fig. 2).

Facilitation of local potentials. With a moderate dose of eserine (0.5–1.0×10^{-5}) in the frog, a single nerve volley causes little prolongation of the endplate negativity, while two volleys following one another at a short interval have a large effect (cf. Fig. 2, 4*A*). This "facilitation" of the local potential is only observed if the volley interval is less than 20 msec.; with greater intervals the second volley produces *less* endplate negativity than the first. In the cat (with a moderate dose of eserine, *e.g.* 100 μg. per kg.) there is an even more striking "facilitation" of local potential (Fig. 3), but as shown in Section III below, this is due to the cumulative action of nerve after-discharges which are produced with two successive volleys at a short interval, but not with one. When there is no after-discharge, the cat differs from the frog in that the second volley always produces less endplate negativity than the first.

3. *Evidence for two separate potential waves.* In many experiments there was evidence that the endplate negativity in eserinized muscle is made up of two successive waves of depolarization, (i) a lengthened e.p.p. decaying

within 50–100 msec., and (ii) a separate "slow wave" of delayed rise and extremely slow decay (Fig. 3, 4). The slow component was brought out most markedly by (i) a relatively large dose of eserine (10^{-5}) and more in the frog, 0.5 mg. per kg. in the cat combined with (ii) a burst of repetitive nerve volleys in rapid succession.

The size of the slow wave varied in different experiments. With a dose of 0.5 to 1.0 mg. per kg. in the cat it reached as much as 40 per cent of the

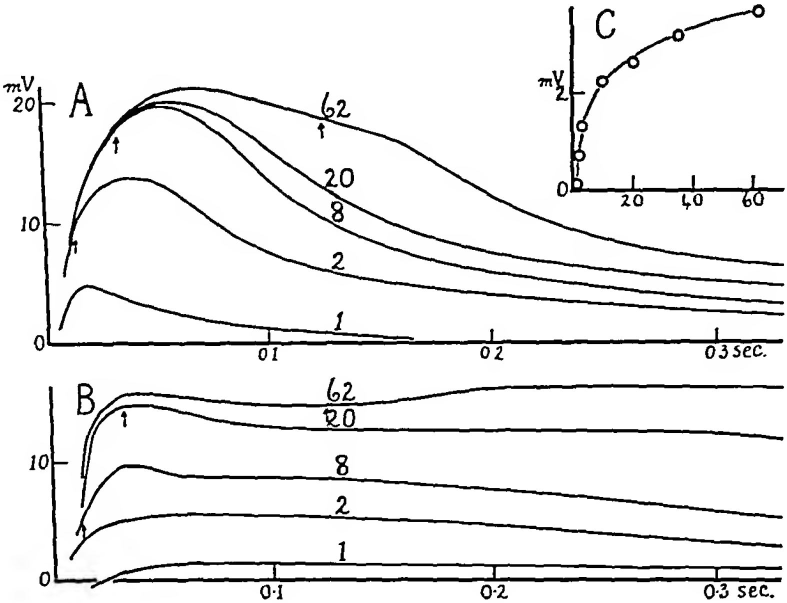

FIG. 4. Frog's sartorius. 22°C. Endplate negativity due to repetitive nerve stimulation (A.C. at 250 c.p.s.). Curves smoothed by disregarding small diphasic spikes; amplifier distortion negligible. Number of stimuli shown in fig; Arrows show end of stimulation periods with 8, 20 and 62. *A:* Eserine 10^{-5}; *B:* Eserine 3×10^{-5}; *C:* Similar experiment, 3×10^{-5} eserine. Ordinates: Height of potential, measured at 0.4 sec. after the middle of the stimulation periods. Abscissae: number of stimuli.

spike potential. At this stage a point of maximum depolarization seemed to have been reached, since it was not increased by additional nerve volleys. The separation between e.p.p. and "slow wave" was never complete, and often was only indicated by an initial phase of decline passing over to a prolonged plateau (Fig. 4*B*). Sometimes, especially with large doses of eserine, the two potentials merged completely. In the cat single or double nerve stimuli gave a large "slow wave" only when accompanied by nerve after-discharges (Section III).

In the frog, where the nerve after-discharge is absent, the slow wave could be built up by repetitive stimulation of the motor nerve. This was done by a sine-wave oscillator (250 c.p.s. giving 2 msec. intervals between alter-

nating stimuli) and the number of stimuli was varied from one or a few up to about 60.

In a normal nerve-sartorius preparation the muscle responded with large propagated spikes throughout the period of stimulation, and little or no endplate negativity was built up (see also 22). In the eserinized preparation, the propagated spike response rapidly declined during continued stimulation (22), and was replaced by a large endplate negativity with sub-

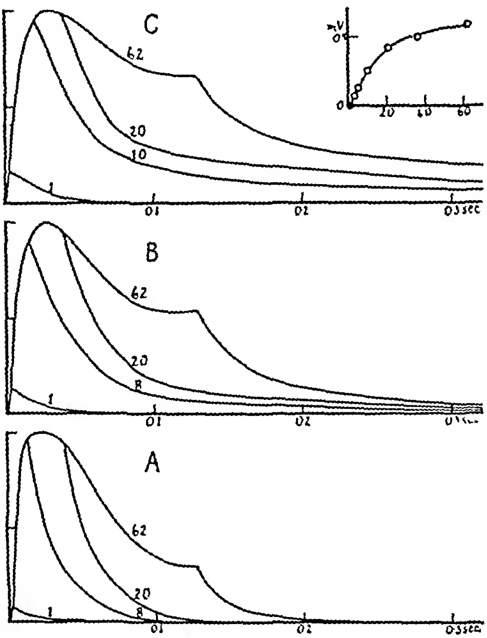

FIG. 5. Frog's sartorious. 24°C. Endplate negativity due to repetitive nerve stimulation, as in Fig. 4. Ordinates in relative units. *A*: 9 μmol. curarine, maximum potential 4.6 mV.; *B*: 9 μmol. curarine +eserine 10^{-5}, max. pot. 5.5 mV.; *C*: 9 μmol. curarine +eserine 2×10^{-5}, max. pot. 3.5 mV. *Inset:* Height of potential in *C* plotted against number of stimuli, at 0.25 sec. after middle of stimulation periods.

maximal spike discharges occasionally superimposed on it. As in the cat, there was clear evidence in several cases for the presence of two waves, an initial phase of relatively quick decline followed by a slow wave which was built up by the repetitive stimulation. A "trough" between the two waves was seen in some experiments, at 60–100 msec., the second maximum occurring at about 0.15–0.2 sec. after the beginning of stimulation (Fig. 4*B*).

It might be asked whether the "trough" was an artefact due to the muscular contraction, which might conceivably bring a region of less endplate negativity in contact with the recording lead. This was excluded because the trough was not affected by small movements of the recording electrode. Moreover, the evidence described below confirms the presence of a late potential change, even when a second rising phase could not be seen.

The relative size and time course of the two components varied somewhat in different muscles and depended upon the dose of eserine and the number of applied nerve stimuli (Fig. 4). Furthermore, with eserine doses

greater than 10^{-5}, the size of the endplate negativity became progressively smaller, while the duration of the slow wave became even greater—up to several seconds with eserine 5 to 10×10^{-5}.

In some experiments on the frog, a double potential rise was observed at rather short intervals (cf. Fig. 9 in Eccles, Katz and Kuffler, 14) with a "trough" at 5 and second maximum at 30 msec. It is not certain whether this effect was really analogous to the much slower second wave described above; it may well have been due to a combination of two potentials, one arising at endplates immediately at the recording lead, the other spreading electrotonically from an adjacent endplate focus (cf. Fig. 2).

There is evidence that with repetitive stimulation the local potentials produced by each successive nerve volley change in a systematic way. There

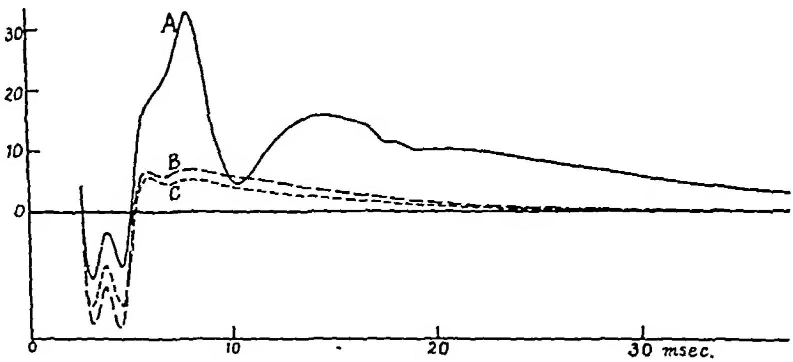

FIG. 6. Cat soleus. Action potentials at endplate zone to a single nerve volley, initial spike not shown. Ordinates: potentials as percentage of spike height. *A*, 300 μg. eserine per kg. *B* shows diminution in e.p.p. after addition of 0.1 mg. curare per kg., the potential closely resembling *C*, 50 μg. eserine per kg., no curare.

is increase with the first few volleys, then progressive decline to less than the initial size (43, 22). As a consequence the pure e.p.p. in curarized muscle was built up to a maximum with the eighth or tenth volley (in 16–20 msec.) and declined with continued stimuli (Fig. 5*A*). Similarly, the size of the slow wave in eserinized muscle, after being greatly increased by the first few volleys, received progressively smaller contributions by the later nerve volleys (Fig. 4*C*). The duration of the slow wave is so long that it has little time to decay during the relatively short period of stimulation (less than 0.12 sec.); hence, in contrast with the e.p.p., it continues to sum even with more than 50 nerve volleys, its size being roughly proportional to the integrated action of the whole series of preceding nerve volleys. During prolonged stimulation, therefore, the initial component of endplate negativity becomes progressively smaller, while the slow wave is built up to a large plateau.

B. Curare-eserine antagonism

A small, subparalytic dose of curare, if applied to a fully eserinized muscle, greatly diminishes the prolonged endplate negativity (*e.g.* Fig. 6).

With further application of curare, the eserine effect is progressively diminished until a pure e.p.p. is left, not differing strikingly from that of eserine-free muscle. Detailed investigation has shown that curare has 3 actions: (i) reducing and eventually obliterating the "slow wave" component; (ii) reducing to a lesser extent and shortening the initial e.p.p.; (iii) in the cat abolishing the after-discharge from the nerve terminals and so completely removing the striking "slow wave" effect in Fig. 3 and 1*C*.

The simplest way of studying curare-eserine antagonism is to apply graded doses of eserine to a completely curarized muscle. With a given concentration of curare (*e.g.* 6 μmol. per l. curarine in the frog), 10^{-5} eserine increased the size and duration of the e.p.p. (Fig. 7). The initial rate of rise is practically unaltered, but the e.p.p. continues to rise to a summit about 100 per cent higher and 2 to 3 times later, from which it falls somewhat more slowly than in the eserine-free muscle. This effect differs significantly from that of other agents, *e.g.* of calcium (4 to 5 times normal content), guanidine (concentration 1/50,000) or partial withdrawal of curarine (Fig. 7), all of which increase the size of the e.p.p. without appreciably altering its time course.

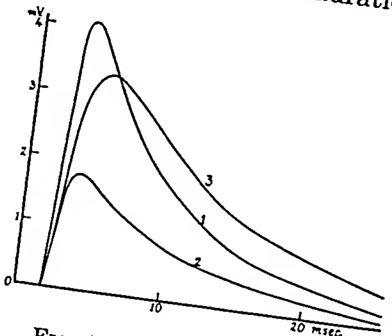

FIG. 7. Frog's sartorius. 23°C. E.p.p. incurarized muscle. *1:* after 6 μmol. curarine; *2:* after 9 μmol. curarine; *3:* after 9 μmol. curarine + eserine 10^{-5}.

The declining phase of the e.p.p. in curarized muscle has been shown to be largely a passive electrotonic decay conditioned by the electric time constant of the muscle membrane; the transmitter action is apparently much shorter than the e.p.p. which it produces (15) and decays effectively in a few milliseconds. The prolonged rising phase of the e.p.p. shows that eserine has increased the duration of the transmitter action about threefold (32). The unaltered rate of rise indicates that there is no appreciable change in the initial intensity of the transmitter action, the increase in the e.p.p. merely being due to the longer duration of this action.

When the eserine dose was increased beyond 10^{-5}, the size of the e.p.p. became smaller. At the same time its time course usually showed a slight further slowing, a maximum lengthening being reached with eserine 2 to 3×10^{-5}. Eserine had less action on the decaying phase of the pure e.p.p. than on its rising phase. Thus the average time for half decay was lengthened by about 50 per cent, *e.g.* from 8 to 12 msec. at 20°C., frog; from 3.5 to 5 msec., cat. There are three possible causes of this lengthening: (i) an effect of eserine on the electric properties and, therefore, on the time constant of the muscle membrane; (ii) a slowing resulting from the greater duration of the rising phase and a consequently greater electrotonic spread of the e.p.p. (cf. 31, p. 300); (iii) a small remainder of transmitter activity persisting during the decay of the e.p.p. and actively maintaining some depolarization.

Factors (i) and (ii) could be eliminated by the following experiments: (i) A simple index of the electric time constant of the muscle membrane was obtained by passing a small, subthreshold current through the muscle *via* 2 non-polarizable electrodes, and recording the time course of the polarization potential (32). Its average half-time is of the same order as that of the e.p.p. in eserine-free muscle (cf. also 44). Eserine had no effect on the time course of this polarization potential, showing that the electric time constant of the membrane is unaltered. (ii) According to the theory of electrotonic potential spread, some slowing of decay would occur as a consequence of the prolonged rising phase of the e.p.p. It is difficult to predict whether this effect would be large enough to account for the observed change. However, one can effectively lengthen the rise of the e.p.p. in curarized eserine-free muscle by applying several nerve volleys in rapid succession. More than a threefold lengthening of the rising phase (4 to 8 shocks at 2 msec. intervals) produced only a 10 per cent slowing of decay.

Thus, it seems most likely that in eserinized muscle the slowed decay of the e.p.p. is largely due to a small remainder of transmitting agent lingering on for many milliseconds and actively supporting the depolarization.

Neuromuscular facilitation after eserine. If the second e.p.p. exceeds a critical threshold level, the arrival of two nerve volleys in curarized muscle gives rise to propagated muscle spikes (15). In frog's muscle, this facilitation process depends upon two factors: (i) summation of successive e.p.p's, (ii) a relatively long "supernormal" period during which the second e.p.p. is greater than the first. Eserine, while increasing the duration of the e.p.p., reduces this "supernormal" effect. To quote the most striking case; with eserine 10^{-5} the maximum second e.p.p. was only 25 per cent greater than the first (as compared with an excess of 92 per cent before eserine); with eserine 2×10^{-5}, the second e.p.p. was only 6 per cent greater than the first. This diminished facilitation offsets the lengthening, and as a net result the duration of neuro-muscular facilitation remains practically unaltered by eserine, as was found by Bremer and Kleintjens (4). With the cat eserine produces no change in the relative size of the second e.p.p. (about 80 per cent, Eccles, Katz and Kuffler, 15), so facilitation is observed with slightly longer volley intervals, *e.g.* 8 msec. increased to 10 msec. Even this small lengthening of facilitation was not found by Maaske, Boyd and Brosnan (37) in dogs paralyzed by curare or magnesium.

It may be of interest to note that in the frog, after eserine, longer intervals between two successive nerve volleys were required to obtain a maximum second e.p.p. Thus, in the above-mentioned experiment, the maximum addition was obtained: (i) with curarine only, after 3 msec.; (ii) with eserine 10^{-5}, after 8 msec.; (iii) with eserine 2×10^{-5}, after nearly 20 msec.

The time course of the second *e.p.p.* was in many cases identical with that of the first; in some frog experiments, however, the second e.p.p. was more markedly lengthened than the first.

In a few experiments on the temperature coefficient of the e.p.p. (15) it was noticed that the effect of eserine (1 to 2×10^{-5}) in lengthening the e.p.p. was abolished by lowering the temperature. This observation will require further study, but it is clearly related to a previous statement by Feng and Shen (26), *viz.* that the localized contraction in eserinized muscle disappears at low temperature.

II. EXCITATORY AND INHIBITORY ACTIONS OF PROLONGED ENDPLATE NEGATIVITY

Previous work on nerve and muscle has shown a close relation between changes of membrane potential and of excitability. It has been pointed out

(14, 15) that the effects of local depolarizations of the muscle at and around the endplate are analogous to those of cathodal currents applied artificially to nerve or muscle fibres. In a normal resting fibre the local depolarization has an excitatory effect: if exceeding a critical level, it can sum with subthreshold electric stimuli (3) and thus, for example, it speeds up the propagation of a muscle impulse (15). In refractory muscle an added e.p.p. was shown to delay the recovery process (16, 17) an effect which is probably responsible for Wedensky inhibition and analogous to cathodal effects in refractory nerve (3, 8). Furthermore it is well known that an intense and prolonged catelectrotonus blocks the propagation of impulses. The increased and prolonged local potentials after eserine would, therefore, be expected to have two opposing effects: (i) production of repetitive muscle spikes; (ii) prolongation of the local refractory period and possibly even block both of neuro-muscular transmission and of the propagation of muscle impulses. These effects have been described by several authors (5, 6, 11, 14, 17, 22, 24, 25, 42) and need not again be dealt with at length.

If a burst of repetitive volleys at 2 msec. intervals is sent into a curarized muscle (cf. Section I*A* above) the e.p.p. builds up to a peak after about 10 volleys and then declines to a much lower level, falling to about 30 per cent of the maximum in 0.12 sec. (Fig. 5*A*). At the end of prolonged stimulation the e.p.p. decays a little more slowly than after a single volley (*e.g.* 35 per cent slowing with 0.1 sec. stimulation). There was no definite evidence for a protracted "slow wave" though a trace of it, not exceeding 1–2 per cent of the e.p.p., was noticed following 0.12 sec. stimulation.

With progressive doses of eserine, a conspicuous "slow wave" component was brought out by repetitive stimulation. Its time course of decay, and rate of summation were much the same as in non-curarized muscle (cf. Fig. 4 and 5), but its size was reduced by curare to a greater extent than that of the initial e.p.p. A number of further experiments were made in an attempt to clear up the nature of the curare-eserine antagonism: their description will be included in the general discussion below.

It appears that any procedure which has deteriorating effects on the muscle membrane (*e.g.*, application of KCl, insufficient rests between stimulations) favours the appearance of "inhibitory" actions (that is effect (ii) above) and reduces or abolishes repetitive response. On the other hand, it was shown by Cowan (11) that those procedures which slow the rate of "accommodation" of frog's muscle (Ca-withdrawal, prolonged cooling of the animals) favour the appearance of prolonged repetitive discharges. This also agrees with the evidence derived from electric stimulation of isolated nerve (30).

(a) *Repetitive muscle responses.* Repetitive muscle spikes arising from the prolonged endplate negativity were frequently observed, both in the cat and frog. It is true that, in the cat, many of these repetitive spikes were associated with repetitive discharges in the motor nerve (Section III). However, simultaneous recording from nerve and muscle (Fig. 8*A*) showed that, even in the absence of nerve after-discharge, the e.p.p. following the spike gave rise to muscle impulses if it was more than about 15 per cent of the normal spike potential (Fig. 1*B*). Moreover, when the slow wave was

separated by a distinct "trough" from the initial e.p.p., the second rise of the potential was associated with a new burst of independent muscle impulses (Fig. 9*B*), which followed a silent period of about 30 msec. during the trough, and were usually unaccompanied by nerve discharge. The initial repetitive response varied considerably in different muscles: *e.g.* tibialis anticus and peronaeus tertius (Fig. 8*B* and *C*) gave a longer train of repetitive responses than soleus (Fig. 1 and 9*A*).

In the frog repetitive discharges quite similar to those in cat muscle were frequently observed, although there was no after-discharge in the motor nerve (Section III; 23). With successive stimulations the repetitive bursts declined in size and duration, while the local potentials were not appreciably diminished. In several cases, a slight injury such as produced by making the muscle "monophasic" (potassium application to one end) practically abolished the repetitive response (Fig. 2). Analogous effects have been observed with electric stimulation of nerve or muscle (30, 41), where repetitive response to constant current is greatly affected by any slight damage. It is clear that the appearance of repetitive spikes in eserinized muscle is no reliable index of the persistence of transmitter activity. They depend upon additional factors involving the excitability of the muscle fibres, their rates both of recovery and of adaptation to prolonged depolarization.

(b) *"Inhibitory" actions at the neuromuscular junction.* (i) Lengthening of refractory period by eserine. It has been shown by Eccles and Kuffler (17) that the minimum interval between two successive muscle spikes in the normal cat's soleus is about 2.2 msec. after an initial direct stimulus, and 3.0 msec. after an initial nerve stimulus. The difference was attributed to a lengthening of refractory period of the muscle by the e.p.p. outlasting the first spike (36). This effect is enhanced by the addition of a second e.p.p. early in the refractory period, and also by the application of eserine, which increases the size and duration of the e.p.p. persisting beyond the spike (Fig. 1). Thus after an initial nerve volley, the minimum spike interval in the cat's soleus muscle was lengthened by eserine from about 3.0 to 5.0 msec. The effects were similar in the frog's sartorius muscle.

(ii) The large slow wave in the cat (30 to 40 per cent of the spike potential) is accompanied by complete neuromuscular block. A testing nerve volley sets up neither muscle impulses nor local negative potential. Furthermore, Feng (21) has shown that a prolonged junctional depolarization by continued high-frequency stimulation blocks the propagation of directly excited muscle impulses past the junctional region. Presumably these effects are due to the depressing action of the prolonged intense catelectrotonus at the endplate region, and are analogous to the cathodal block in nerve first described by Werigo (46).

III. REPETITIVE DISCHARGE FROM ESERINIZED NERVE TERMINALS

Masland and Wigton (40) observed repetitive after-discharges in the motor nerve, originating from the nerve terminals of eserinized cat's

muscle. It was of interest to study the relation of these nerve discharges to the local and propagated potential changes in the muscle. This was done by recording simultaneously (i) the potential changes at the endplate region of the innervated strip preparation of soleus or peronaeus tertius; and (ii) the motor impulses in the nerve supplying this muscle, the afferent fibres having been degenerated by aseptic removal of the dorsal root ganglion of the corresponding spinal nerves at least four days previously. The motor nerve was cut at the beginning of the experiment; the recording electrodes were applied more peripherally. Repeated tests during the experiments showed that relatively weak shocks applied through the recording electrodes gave a full muscle response, hence conduction from the nerve terminals to the recording lead was unimpaired in all motor fibres concerned.

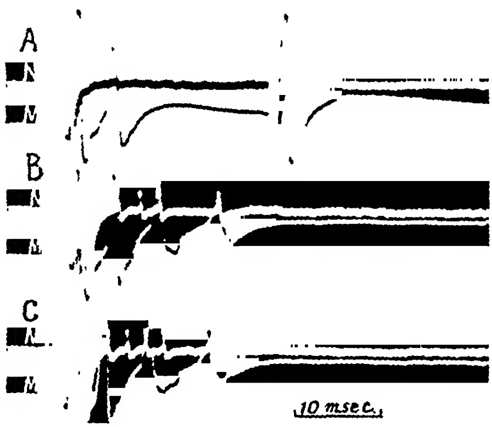

FIG. 8. Cat, peronaeus tertius, 300 μg. eserine per kg. Simultaneously recorded action potentials of endplate zone of muscle (*M*) and deafferented motor nerve (*N*) to two nerve volleys. Initial nerve spikes not shown, as amplification too high. *A:* no nerve after-discharges with volley interval of 24 msec.; *B* and *C:* with volley intervals of 1.2 and 1.6 msec. after-discharges of four and five nerve impulses apparently in same nerve fibre. Note more intense repetitive muscle response and more prolonged negative potential.

The finding of Masland and Wigton (40) was readily confirmed. With increasing eserine dosage, there was in many experiments a striking parallelism between the onsets of repetitive discharges in muscle and nerve fibres. In the absence of simultaneous recording the con-conclusion might well have been drawn that a separate nerve impulse was responsible for every discharge of the muscle. In every experiment, however, some repetitive muscle spikes were observed in the absence of nerve after-discharges (Fig. 1*B* and 8*A*), *i.e.* the prolonged endplate negativity alone is capable of setting up multiple muscle spikes (cf. preceding section). This is quite clear also with eserinized frog muscle, where simultaneous recording from the ventral roots revealed complete absence of nerve after-discharges (confirming Feng, 23).

The cause of the nerve after-discharge remains an open question. With increased eserine dosage, impulses are discharged spontaneously from the nerve terminals (40) and cause the well-known eserine twitching. This suggests that, prior to the onset of spontaneous discharges, the nerve terminals must be in such a hyper-excitable condition that a small additional stimulus would fire them off. This additional stimulus may be provided in various ways: for example, as Masland and Wigton have shown, by an intra-arterial injection of acetylcholine, or as shown below, by the action currents of the muscle.

The retrograde effect of the muscle spike on the nerve terminals is tested by stimulating the muscle directly at some distance from the endplate zone, and recording simultaneously from junction and motor nerve. In normal, or even in unconditioned eserinized preparations antidromic muscle spikes are never found to excite motor nerve terminals. On the other hand, when the eserinized muscle (100 μg. per kg. or more) is conditioned by one or more preceding nerve volleys, an antidromic spike always evokes the discharge of nerve impulses (Fig. 9*A*), thus giving a "retrograde trans-

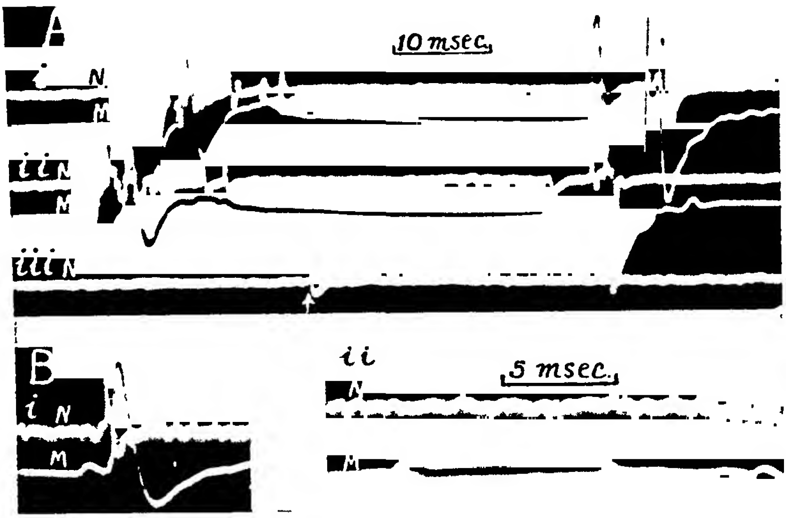

FIG. 9. *A*: Cat, soleus, 200 μg. eserine per kg. Simultaneous action potentials as with Fig. 8. Muscle amplifier has very short time constant, so so "slow wave" not recorded. *i* and *ii*: Initial nerve volley sets up repetitive muscle response and nerve after-discharge (initial muscle and nerve spikes not shown); antidromic muscle volley 49 msec. later (spike partly shown) also sets up brief burst of nerve impulses, beginning about 1 msec. after arrival of muscle spike at endplate zone. *iii*: Nerve record only, showing no nerve discharge due to antidromic volley alone (stimulus artefact shown by arrow).

B: *i*: "triple response" arising 50 msec. after a double nerve volley —small muscle spike followed by nerve spike, then large muscle spike. *ii*: as in *i*, but showing three small muscle spikes without the later nerve and large muscle spikes.

mission" from muscle to nerve. The possibility of nerve excitation by stimulus leak is easily excluded, for the nerve impulses are observed only after the muscle impulses have travelled from their point of origin to the endplate zone.

There is close correlation between the period of "retrograde transmission" and the duration of the negative potential at the endplate region. When a large slow wave is present "retrograde transmission" can be observed for several seconds after the conditioning nerve volley. In the ab-

sence of a large slow wave, reversed transmission is often observed for several (8–14) msec. only, *i.e.* during the decay of the e.p.p.

This "retrograde transmission" suggests that repetitive muscle spikes arising directly from the prolonged endplate negativity (Section II) would be responsible for some of the nerve discharge. Further evidence for this is given by records showing a triple sequence of action potentials (Fig. 9*B*): a small muscle action potential—possibly due to an impulse arising at a single endplate—an immediately following nerve impulse, and then the large muscle spike of that motor unit. There is no doubt, however, that some nerve discharges are independent of, and prior to, the muscle impulses (cf. the initial nerve after discharge of Fig. 9*A*). Whether these nerve spikes are set up by an electric effect, *e.g.* by the concomitant e.p.p., or by acetylcholine action (40) cannot be decided at present.

With moderate doses of aserine (50–100 μg per kg.) two nerve volleys at intervals of less than 10 msec. set up a nerve after-discharge, while there is no such discharge with a single volley. This explains the striking potentiation of endplate negativity and muscle discharges observed in the cat with two nerve volleys at a short interval (Fig. 3, 8). Even if originating at a single nerve terminal only, the repetitive nerve impulses would spread by "axon reflexes" to all the fibres of that motor unit, *i.e.* in addition to the two initial nerve volleys the whole motor unit is subjected to a brief high-frequency burst of nerve impulses. Presumably such "axon reflexes" are responsible for the sequence of small initial and large later muscle spike in the "triple responses" described above (Fig. 9*B*).

Discussion

The observations in this paper are consistent with the view that the junctional region of the muscle responds to the local depolarizations in the same way as any other region of the muscle responds to a catelectrotonic potential. If there is any special process at the junctional region, it would concern the setting up of the catelectrotonus by the transmitting agent, and it is this process which is lengthened by eserine, and diminished and shortened by curarine.

The fact that eserine lengthens the depolarizing action of the transmitter (see Section I*B* above) focusses attention on the acetylcholine (ACh) theory. It seems likely that this effect is related to the inhibition by eserine of ACh hydrolysis, and hence that ACh is the agent responsible for the junctional potential change. Some difficulties, however, arise when considering the curare-eserine antagonism (Section I*B*).

Further experiments on curare-eserine antagonism. Curare is known to oppose the depolarization of the muscle membrane by ACh (10); hence the diminution of the e.p.p. by curare provides no obstacle to the ACh hypothesis. On the other hand it is difficult to explain the drastic shortening by curare of the prolonged eserine-potential (shortening of e.p.p. and great

diminution of slow wave). The following possibilities have been tested experimentally.

1. In order to detect a possible antagonism between curarine and eserine with regard to esterase inhibition, the hydrolysis of ACh by muscle and serum esterase was measured by the manometric method (1, 45).

The manometers were filled with N_2 containing 5.1 per cent CO_2. Each vessel contained a known volume of bicarbonate Ringer (using the mixtures described by Stedman and Stedman, 45, and Marnay and Nachmansohn, 39, for dilution of human serum and frog's tissue suspensions respectively) to which was added a known volume of an enzyme preparation—human blood serum, or a fine suspension of frog's nerves or innervated portions of sartorius and semitendinosus muscles. In the example of Fig. 10, muscle suspensions were used, each flask containing 170 mg. in 3 ml. solution. The contents of the 3 flasks were made up as follows: *A*, Muscle in 3 ml. bicarbonate Ringer; *B* as *A*, but containing eserine 10^{-5}; *C* as *B*, but containing in addition 7.5 μmol./l. curarine. The side bulb contained 0.3 ml. of 3.7 per cent solution of acetylcholine (B.D.H., London) neutralized before use. This ACh was added to the flask solution about one hour after the filling of the flasks.

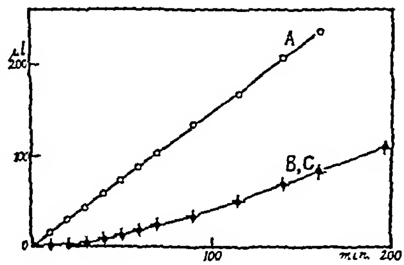

FIG. 10. Manometric determination of esterase activity. Enzyme preparation: Suspension of innervated portions of frog's muscles (170 mg. in each flask), at 15°C. Ordinates: CO_2—production in μl.; abscissae: time in minutes. *A*: normal Ringer; *B* (crosses): Ringer containing 10^{-5} eserine; *C* (full circles): Ringer containing 10^{-5} eserine $+7.5$ μmol. curarine.

It is clear from Fig. 10 that the addition of curarine (concentration 7.5 μmol. per l.), in no way influenced the rate of hydrolysis by the partially inhibited enzyme preparation (eserine concentration 10^{-5}).

Figure 10 shows that the activity of the eserinized enzyme solution increased gradually to a study rate after the substrate has been added, while the uninhibited enzyme acts at constant rate. Presumably this difference is due to a "competition" between inhibitor and substrate, *i.e.* to a gradual displacement of eserine from the eserine-enzyme compound by the added ACh, until a steady state is reached (13). Incidentally the results show that curarine in concentrations more than sufficient to block transmission does not inhibit the enzyme activity. With high concentrations (20 μmol. per l.) it was found to increase the eserine inhibition by about 25 per cent. This contrasts with a recent observation by Harris and Harris (27) who reported a marked inhibition of cholinesterase by a curare preparation "Intocostrin"; probably some substance other than curarine was responsible for this effect.

In two experiments intact muscles were soaked in 2 ml. of an ACh Ringer solution (1.5×10^{-5}) containing either eserine (1 to 2×10^{-5}) or eserine $+$curarine (9 μmol. per l.). After 3 to 4 hours at 18°C., the solutions were tested for ACh content by local application of a small quantity to a chronically denervated frog's sartorius which responded to ACh concentrations of less than 10^{-8}. There is no significant difference in the activities of the two solutions (*i.e.* less than a change of 2:1). It may be recalled that Dale, Feldberg and Vogt (12) found no appreciable change in the ACh

activity of perfusates of stimulated nerve-muscle preparations before and after curarization.

It can be concluded, therefore, that curarine does not antagonize the inhibition of cholinesterase by eserine.

2. Curare might curtail the endplate negativity by somehow quickening the rate of "accommodation" to the drug action. This was tested by recording the discharge of impulses in the frog's sartorius muscle following local application of ACh.

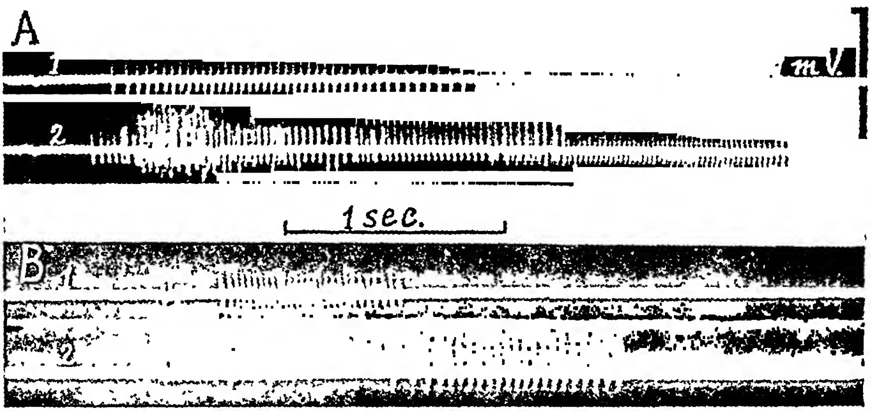

FIG. 11. Frog's sartorius. 16°C. Response to applied ACh. *A*. normal muscle. *1:* ACh 3×10^{-6}; *2:*ACh 10^{-5}. No discharges with ACh 10^{-6}. *B*. After 6 μmol. curarine. *1:* ACh 3×10^{-5}; *2:* ACh 10^{-4}. No discharges with ACh 10^{-5}.

The solutions were applied to the surface of the muscle by a platinum loop containing about 0.3 μ l. of solution and making contact with about 1 mm. of muscle length. The threshold concentrations of ACh varied from point to point, depending presumably upon the presence of superficial neuro-muscular junctions in close vicinity to the point of application. Invariably, however, the sensitivity to ACh was much lower at the nerve-free pelvic end than in the innervated parts. After a dose of curarine blocking neuromuscular transmission (3 to 6 μmol. per l.) the threshold concentrations for ACh rose 10 to 100 times, in contrast to those for KCl which were little, if at all, increased. For a given superthreshold concentration of ACh (*e.g.* 3 times that required to set up one or a few single fibre impulses, the duration of the repetitive discharge was sometimes a little shortened by curarization, Fig. 11; cf. Blair, 2). Delayed responses, and low-frequency discharges were still obtained. These observations show that, if there is any quickening of "accommodation" to ACh by curarine, it would be quite inadequate to account for the very drastic shortening of the eserine potential. In contrast with this, high-calcium Ringer (5 times the normal content), which is known to speed up accommodation to electrical and other stimuli, not only raised the threshold concentration, but reduced the discharge to one or two initial impulses and abolished any delayed response.

3. It might be that the prolonged eserine potential, particularly the slow wave, is not due to continuing ACh action, but is similar in nature to the prolonged negative after-potential such as is produced by veratrine. It is difficult to rule out this possibility entirely, but it must be remembered that the prolonged eserine-potential is restricted to the junctional region and that it is not produced by antidromic muscle impulses. Veratrine in low concentrations (2 to 5×10^{-8}) was found to enhance and prolong the negative after-potential of muscle (duration 2 sec. or more, size up to 20 mV) and cause repetitive responses, but it did not alter the size and time course of the e.p.p. due to an early second nerve volley.

It appears that some other explanation of the curare-eserine antagonism has to be sought. As outlined below all the observations of the present paper are compatible with the ACh hypothesis, if certain extensions of this hypothesis are made.

The facts on which the ACh hypothesis is based, are well-known: ACh is liberated by motor nerve impulses (12); it has a powerful and rapid stimulating action on the endplate region of the muscle (5, 6, 7); it is rapidly hydrolyzed by an enzyme concentrated at or near the endplates (39). The rapidity and chemical specificity of the ACh effect suggest that ACh produces its depolarizing action by combining with specific chemical receptors on the surface (9). Curarine, while apparently not affecting the ACh liberation, opposes its depolarizing action on the muscle membrane (10). Since, in addition to this specific blocking action, curarine chemically resembles ACh (both are quaternary ammonium cations, as also are other substances with similar actions, cf. Ing, 29), it seems likely that it also acts by combining with these same chemical receptors (cf. the receptive substance of Langley).

Eserine combines with the active centres of the cholinesterase molecules (13) and so blocks their hydrolyzing action on ACh. In addition eserine may have a weak chemical affinity to the muscle receptors, as is suggested by the fact that large doses of eserine diminish the endplate depolarization (Section I*B*).

If cholinesterase is highly concentrated at the endplate region (39), it would have a "barrier" effect preventing ACh from spreading to, and stimulating, adjacent muscle and nerve membranes. It has even been suggested (20, 38) that this may be the main or sole function of the esterase, and that the removal of ACh at the site of its release may be accomplished in a different way, *e.g.* by resynthesis to a chemical precursor.

In curarizied muscle even in the presence of large doses of eserine the duration of e.p.p. and the underlying transmitter action is rather brief (Section I*B*), indicating that the removal of the transmitter still proceeds at a high rate. Some eserine-resistant mechanism of destruction must be present, whether it be due to a chemical restitution as suggested above, or to some cholinesterase accessible to ACh but not to eserine. Further in explaining the slow wave (cf. below) the additional assumption is made that this mechanism is strictly localized to the nerve-muscle junction, as indeed would be the case if it were a restitution process.

The "slow wave" set up in eserinized muscles (Section I*A*) indicates that there is a separate delayed action of the transmittter while its initial effect is declining. The relatively rapid spread of substances along interfaces to which they have chemical affinity (28) provides a possible explanation of this. ACh combining with membrane receptors would spread along the muscle surface from receptor to receptor and involve an increasing area of muscle membrane adjacent to the endplate. In the absence of eserine, this spread is prevented by the enzyme "barrier." In eserinized muscle, while ACh may still be inactivated at the endplate by a rapid, eserine-resistant mechanism (see above), some of it can spread beyond the range of this destruction and so give rise to the delayed slow wave. With a small dose of eserine, a single nerve volley is not sufficient to produce a slow wave. Summation of two or more nerve volleys is required (Section I*A*): the first few volleys help to maintain saturation of the remaining active enzyme molecules, while the successive quantities of ACh then can spread beyond the "barrier." Small doses of curarine greatly reduce the slow wave (Section I*B*). This might be attributed to the combination of curarine with the surface receptors (see above); curarine would diminish the rate of surface spread of ACh by blocking its combination with the receptors. Moreover, in this way curarine would

increase the chances of free ACh combining with active enzyme molecules and so enhance somewhat the rate of hydrolysis. Presumably this would account at least partly for the shortening of transmitter action by curarine in both eserinized and eserine-free muscle (Section I*B*; and 17).

Thus by making plausible assumptions, the observed curarine and eserine actions may readily be reconciled with the hypothesis that ACh, liberated by nerve impulses, is responsible for all the local potential changes at the neuromuscular junctions.

Summary

Both in cat's and frog's muscles eserine increases and lengthens the local negative potential change (the endplate potential, e.p.p.) set up by one or more nerve volleys at the myoneural junction.

In addition, repetitive nerve volleys produce a delayed "slow wave" (height up to 40 per cent of spike, duration up to several seconds) at the junctional region of eserinized muscle. The onset of the slow wave merges more or less with the decaying phase of the e.p.p.

Curare antagonizes the eserine effect: it greatly reduces the slow wave component, and shortens and diminishes the e.p.p. Even in fully curarized muscle, eserine causes a prolongation of the e.p.p.; the rising phase is lengthened up to threefold, the e.p.p. thus building up to a greater height. Both in normal and curarized muscle increase of eserine beyond a certain high concentration (about 3×10^{-5} in the frog) causes no further prolongation of the negative potentials, and actually diminishes their size.

The junctional potentials of eserinized muscle give the typical effects of an increased and prolonged catelectrotonus: (i) repetitive muscle spikes, when above threshold size; and (ii) lengthening of refractory period, and, during very intense depolarization, a complete block of impulse propagation.

The production of nerve after-discharge in the eserinized cat has been further investigated. Repetitive muscle impulses can arise from the junctional potentials independently of nerve after-discharge. In the cat, during prolonged eserine potentials, retrograde propagation of impulses from muscle to nerve was observed.

The principal effect of eserine is a lengthening of the action of the neuromuscular transmitter, thus leading to prolonged junctional negativity with consequent catelectrotonic effects.

In an attempted analysis of the curare-eserine antagonism it is shown that (i) curare does not antagonize the inhibition of cholinesterase by eserine; (ii) curare has little or no effect in quickening the adaptation of muscle to acetylcholine.

By making plausible assumptions it is shown that the observed curare and eserine actions are reconcilable with the hypothesis that ACh is responsible for all the local potential changes set up by nerve impulses.

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EXCITABILITY OF CEREBRAL CORTEX IN INFANT *MACACA MULATTA**

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UNTIL THE second month, when voluntary movements begin, the motions of the infant monkey (*Macaca mulatta*) suggest that its cerebral cortex has little function. Some it must have, however, for cortical ablations cause some alterations of movement, and stimulation of area 4 at that age elicits some movements. Such responses to stimulation by induced currents from a Harvard coreless inductorium have already been reported from this Laboratory (3), and responses to 60-cycle stimulation of the infant cortex have been minutely investigated by Hines and Boynton (2) at various stages from fetal life to the end of the first year.

However, facilitation, extinction and suppression of motor response to cortical stimulation had never been studied in the infant monkey. The type and location of stimulation required to demonstrate them in the adult monkey are well known (1). Briefly, at any motor focus repetition of appropriate stimulation at short intervals yields facilitation; at longer intervals, extinction; and stimulation of any suppressor area (*e.g.* 4-s or 8) prevents response to subsequent stimulation of all motor foci. Those foci for specific discrete responses occupy (Brodmann's) area 4, and those for complex responses lie in area 6.

Since these characteristics have been demonstrated unequivocally in a single experiment upon a monkey 20 days old, the results appeared worth reporting. They are the more significant because the infant was normal for its age in weight, behavior and in the electroencephalogram. It exhibited normal righting reflexes, stood and walked on a wide base. It clung, climbed and sucked readily, and exhibited forceful and sustained reflex grasping as soon and as long as the palm or sole was touched. Its EEG before anesthesia showed typical low-voltage waves at 4–5 per sec.

PROCEDURE

A twenty-day old infant monkey (*Macaca mulatta*), wt. 515 gm., was fully anesthetized with 0.16 cc. Dial (Ciba), one-half intraperitoneally and one-half intramuscularly, and its cortex exposed for bipolar stimulation. Electrical pulses of controlled wave form, frequency and voltage were delivered by a stimulator having an output unaffected by the resistance between the electrodes. Motor responses were noted by three observers. Subsequently the cortex was locally strychninized and the induced electrical disturbance of the cortex recorded from bipolar leads, with a six-channel Grass electroencephalograph.

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Results

Areas yielding motor response. Bipolar electrical stimulation, even with impulses of abrupt rising phase and ten msec. falling phase, of relatively high voltage and of any frequency and duration, elicited motor responses only from the posterior margin of area 4—not from its anterior portion, nor from area 6, nor from the postcentral convolution. Suppression of these motor responses was produced, with the usual latency of several minutes, by stimulation of area 8 above the sulcus arcuatus (Fig. 1, pt. 51) and by

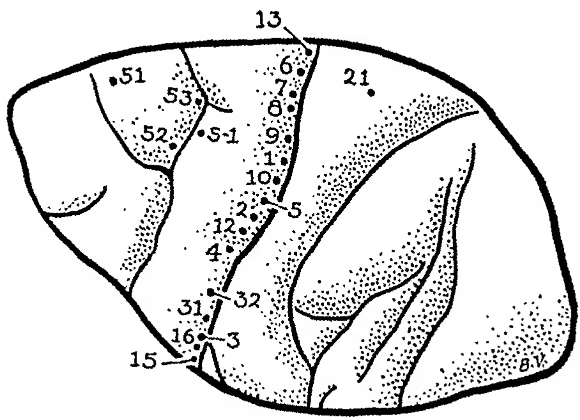

FIG. 1. (*Macaca mulatta*) operative exposure left hemisphere 20th day infant. For symbols, see text.

stimulation of area 4-s (pt. s-1). The locations and sequence of stimulations are given in Fig. 1, and the corresponding responses were as follows:

1. Shoulder, followed by whole arm
2. Thumb adduction, followed by flexion and supination at wrist
3. Tongue and upper lip
4. Thumb
5. At times extension and eversion of wrist, at times extension of thumb
6. No response usually, twice questionable response of shoulder
7. Thigh once, shoulder once
8. Change in respiratory rate, no abdominal contraction
9. Outward rotation of shoulder
10. Forearm and wrist
12. Thumb followed by 2nd and 3rd finger
13. Hamstrings, flexion of leg
15. Tongue and upper lip
16. Tongue and upper lip
21. No response
31. Lower jaw
32. Upper lip
52. No response
53. No response

S-1. Suppression
51. Suppression

Character of response. It should be noted that movements of the proximal portions of the extremities were elicited more easily and regularly than those of the distal parts; that responses of the face were well developed, those of the hand, thumb and finger, much less well defined and those of the foot and toes absent.

Compared with those obtained from the adult, the movements themselves were less constant, less discrete and slower in onset, execution and termination. Moreover, the threshold of motor foci was higher, no responses

could be elicited even with long, high voltage pulses at 1 per sec. and the responses to 5 pulses per sec. were not prompt and separate, as in the adult, but late and fused. This is obviously due to facilitation which was easily demonstrated by repetition of stimulation with short intervals. With intervals of about 15 seconds there was total extinction of motor response.

The threshold for suppression of motor response, both in areas 8 and 4-s, was like that of the adult, and the motor after-discharge provoked by stimulation in area 4 was held in abeyance by application of the same stimulation to area 4-s (Fig. 1. S-1)

Strychninization. Finally, local strychninization of area 4 produced typical strychnine spikes, and strychninization of area 4-s (at S-1) induced a typical suppression of electrical activity of the cortex.

Discussion

The responses to stimulation of the cortex of this three-week old macaque indicate that area 4 is already capable of initiating those slow and ill-differentiated movements which are typical of its age. Nor is it surprising that these are obtained from area 4 which is first to develop and first myelinated. What is of interest is that among the descending systems the shorter (to face and even to hand) are nearer to adult performance than the longer (to leg).

Since facilitation and extinction of motor response have both been demonstrated in the infant cortex, the interpretation of the slow and only relatively discrete motions deserves consideration. Since, as judged by motor response, the threshold is higher, the lack of separate responses to pulses at 1 per sec. and the tardy and fused responses to 5 per sec. may be taken to mean either that synchronous discharges leaving the cortex reach the cord late, scattered and decimated, or that the threshold of the cells of the cortex is itself higher. The latter seems unlikely, for 4-s and 8 have normal thresholds, though they are developmentally behind area 4. If the former is the fact, then one would have to expect less discrete response because of facilitation of neighboring motor foci and perhaps partial extinction of the focus under the electrode. In this case the apparent lack of differentiation of the cortical foci need not imply any inactivity of cortical elements,—merely a difference in the descending system. Be that as it may, the difference in the discreteness of motor response is not an artifact of stimulation, for the infant monkey in using its cortex would encounter the same difficulty. This is confirmed by the conformity of normal activity to that induced by cortical stimulation.

The failure to elicit motor responses from area 6 in the infant which still exhibits forced grasping is in harmony with the results of removal of area 6 in the adult, which brings on forced grasping.

That removal of 4-s at any age gives rise to spasticity only after the first six months of life, and that excitation of 4-s even in the third week gives suppression, suggest that the as yet unknown structure whose unsuppressed activity would yield spasticity is not yet active in the infant.

SUMMARY

The cerebral cortex of the three-week old (infant) *Macaca mulatta,* when stimulated electrically, produces movement contralaterally.

The stimulable motor foci are confined to area 4 and no responses are elicitable from area 6 or the postcentral convolution.

Facilitation and extinction of motor response are easily demonstrable.

Relatively high voltages are required to elicit responses and these, like the spontaneous motions, are less discrete and slower in onset, execution and cessation than those of the adult. This may indicate either higher threshold in an as yet undeveloped cortex or less adequate corticospinal conduction.

Responses are more easily elicited in the face, less in the hand and not at all in the leg.

Lack of response from area 6 is correlated with the forceful, sustained and easily elicited forced grasping normal for that age.

That suppression of motor response is elicitable from 8 and 4-s at this age when removal of 4-s does not yet produce spasticity suggests the functional immaturity of that structure whose unsuppressed activity would give rise to spasticity in older animals.

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EFFECTS OF PRESYNAPTIC VOLLEYS ON SPREAD OF IMPULSES OVER THE SOMA OF THE MOTONEURON

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INTRODUCTION

RECENTLY the nature of impulse conduction at the soma (cell body and dendrites) of the neuron has been subjected to direct experimental examination (5, 6). The potential changes which may be recorded at a small needle electrode placed in a cranial motor nucleus reveal that stimulation of a motoneuron produces, in addition to the nerve impulse which sweeps over its axon, a response which must be attributed to the soma. This response is approximately the same whether the motoneuron be stimulated synaptically or antidromically. An impulse initiated in the axon or at some portion of the soma must therefore spread, at least to a certain extent, over the soma. Lorente de Nó has also shown that the electrical sign of activity at the soma is decreased in the successive responses to a rapid series of antidromic volleys. Asphyxia blocks passage of the antidromic impulses from the axons into the somas of the motoneurons.

The present experiments reveal that responses of the somas of spinal motoneurons resemble those of cranial motoneurons. It is also shown that the electrical response of the motoneuron soma may be augmented or, as the case may be, depressed by the central effects of sensory volleys. Even conditioning dorsal root volleys which fail to set up impulses in the axons of the tested motoneurons suffice to produce these alterations in the character of conduction at the soma.

METHODS

The experiments were carried out on rabbits and cats which had been either decerebrated or lightly narcotized with pentobaribtal sodium ("Nembutal"). After a laminectomy had been performed and the necessary spinal roots prepared, the cord was covered with a layer of paraffin oil. Conditioning afferent volleys were set up by the application of single shocks to groups of dorsal rootlets. Centripetal testing volleys were initiated in groups of motor axons either by stimulation of ventral roots which had been severed as they passed through the dura mater, or by stimulation of the central ends of cut peripheral motor nerves. In the latter case it was necessary to cut dorsal roots to prevent the entrance into the cord of impulses in sensory fibers of the stimulated nerve. Records were made of the potential differences which arose between a micro-electrode inserted into the ventral horn and a second, indifferently placed electrode. The micro-electrodes were steel needles of shank diameter 50 micra. They were insulated with enamel except at their tips, which had been ground at a gradual taper to sharp points. The usual differential amplifier, oscillograph and stimulating apparatus were used.

RESULTS

1. *Response of spinal motoneurons to antidromic stimulation.* The present experiments on motoneurons of the lumbo-sacral cord have confirmed several facts discovered by Lorente de Nó (5, 6) in his studies on the neurons of the

3rd, 6th and 12th cranial motor nuclei. In particular, (i) there is an electrical response of the soma which may be differentiated from that of the axon, and (ii) the response of the soma is much more subject to modification than the impulse (spike potential) in the axon. As shown by Gasser (1, p. 144), the shape of the axonal spike is remarkably constant, and under the conditions of the present experiments can not be expected to show any change.

The illustrative records of Fig. 1 have been taken from an experiment upon a decerebrated cat. After performing a laminectomy and opening the dura, the seventh lumbar (L_7) ventral root and the L_6 and L_7 dorsal roots were cut intradurally. The severed ventral root was raised on stimulating electrodes through which shocks maximal for alpha fibers were delivered. The centripetal motor volleys so initiated produced potential changes which were recorded at two loci.

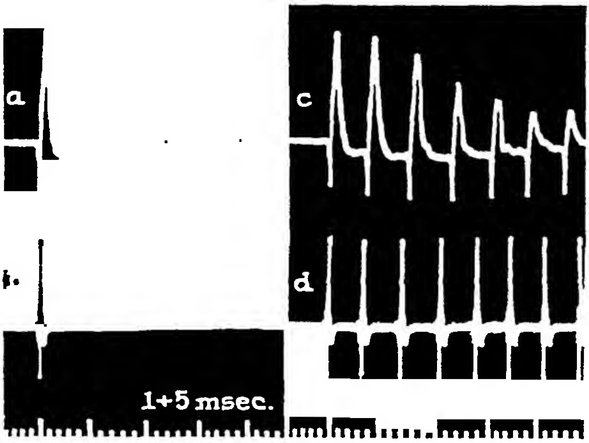

FIG. 1. Comparison of the responses of motoneuron somas (*a*, *c*) and of motor axons (*b*, *d*). Decerebrated cat. Records *a* and *c* were obtained with a micro-electrode in the ventral horn; *b* and *d*, with a small Ag-AgCl ball electrode on ventral root axons. In each case an electrode at a distant point completed the recording circuit. In this and the subsequent figures an upward deflection indicates negativity at the micro-electrode. The stimuli were single (*a*, *b*) and repetitive (*c*, *d*) shocks, maximal for alpha fibers, delivered to the ventral root. The amplification for records *a* and *c* was 5 times that for *b* and *d*.

(i) In the case of records *b* and *d* of Fig. 1 the active recording lead was a small Ag-AgCl ball electrode placed on the ventral root axons between their point of emergence from the cord at L_7 and the distally located stimulating electrodes. At this point the motor axons lay ventrolaterally beside the sacral cord; they were partially surrounded by a conducting medium consisting of the cord, the dura, a small amount of accumulated fluid and the underlying bone of the vertebral column. The rapid triphasic response (record *b*) is characteristic of the sequence of potential changes which are produced in a volume conductor at points close to a bundle of nerve fibers which conduct a nearly synchronous volley of impulses. The motor axons followed the stimulating shocks at high frequencies, for it is seen (record *d*) that successive centripetal motor volleys set up at a frequency of *circa* 286 per sec. were identical.

(ii) A small electrode was inserted into the L_7 ventral horn (records *a* and *c*). The recorded sequence of potential changes was very different from that produced by the conducted spike potentials in the motor axons. In

analogy with the findings of Lorente de Nó, record *a* from the ventral horn must be interpreted as follows. The initial downward deflection, which represented positivity at the micro-electrode, signalled the approach of the centripetal volley of impulses in the motor axons. The large negative (upward) deflection had a duration of somewhat more than 1 msec. It represented a depolarization of the somas of the motoneurons whose axons were occupied by the centripetal volley. Finally there appeared a much smaller and more prolonged positivity which indicated the existence of a difference in potential between the portions of the motoneuron somas near to the origin of the axons and the more terminal ramifications of the dendritic systems, the flow of current taking place in the direction from the cell bodies toward the dendritic tips.

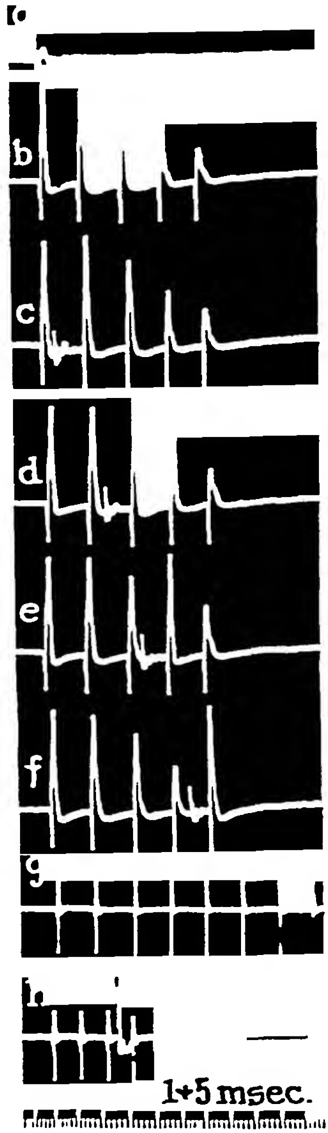

FIG. 2. Effect of dorsal root volley on response of motoneuron somas. Decerebrated cat. The recording micro-electrode was in the ventral horn for records *a-f;* for records *g* and *h* it was inserted deep into the ventral white matter. *a,* L_7 dorsal root volley in isolation; *b,* 5 L_7 ventral root volleys in isolation; *c-f,* the dorsal root volley interpolated during the series of ventral root volleys; *g,* series of L_7 ventral root volleys; *h* interpolation of L_7 dorsal root volley in series of 5 L_7 ventral root volleys recorded from the ventral white matter.

The negative deflection, which signalled the entrance of the antidromic impulses into the somas of the motoneurons, did not maintain its full size in the successive responses to repetitive stimulation. In the case of record *c* of Fig. 1, for instance, the sixth response was reduced to one-third the height of the first. This alteration of the successive responses of the somas was not due to a comparable reduction in the size of the centripetal volleys in the motor axons for, as has been stated, these remained essentially unchanged (record *d*). The response of the soma of the motoneuron is therefore altered, and judging by the size of the negative deflection, is reduced at frequencies of stimulation which its axon is able to follow without difficulty.

2. *Conditioning by dorsal root volleys of the motoneuron response to antidromic stimulation.* The responses of motoneuron somas to stimulation by

centripetal impulses in their own axons are readily altered by the spinal activity due to arrival at the cord of impulses in sensory fibers (Fig. 2–4). In general the central effects produced by a dorsal root volley include both the changes due to activity in the primary afferent neurons themselves and the changes due to stimulation of intraspinal neurons. Depending on the tested motoneurons and on the population of sensory fibers occupied by the conditioning volley, the response of the motoneurons may be facilitated or inhibited; that is, the negativity referable to the invasion of the motoneuron soma by the testing centripetal motor impulses may be either augmented or reduced.

The records of Fig. 2, taken from the same experiment as those of Fig. 1, reveal a striking facilitatory effect of dorsal root volleys on motoneuron responses made subnormal as a result of repetitive antidromic stimulation. The active recording lead for records *a-f* was a micro-electrode inserted into the L_7 ventral horn. Record *a* shows the response to an L_7 dorsal root volley. In record *b* are seen the potential changes which arose as a result of the delivery of five shocks to the ventral root. There was a progressive decrease in the magnitude of the motoneuron response. In record *c* a dorsal root shock was applied during the interval between the initiation of the first and second motor nerve volleys. The responses to the second and third antidromic volleys were then much greater than in record *b*. It is noteworthy that the second response was not only greater than the second response when no dorsal root volley had preceded. It was also slightly but significantly larger than the response to an unconditioned antidromic stimulus, as may be seen by comparing the second response of record *c* with the first response of records *b-f*. In records *d* to *f* the dorsal root shock fell progressively later in the series of ventral root volleys. In each instance the subsequent motoneuron discharges were facilitated. Even the small response of the motoneurons to the fifth antidromic volley was facilitated by the preceding dorsal root volley (record *f*) to a size at least as large as the response of the motoneurons to the first (unconditioned) antidromic volley.

In contrast, the centripetal volleys in the motor axons were not altered in a comparable way. Records *g* and *h* of Fig. 2 were taken after the recording micro-electrode had been pushed deep into the ventral white matter, perhaps even into the bundles of motor axons which underlie the cord itself. The recorded responses therefore represent mainly the activity of motor axons. It is seen that, in contrast with the responses of the somas, the triphasic axon spike potentials remained constant during repetitive stimulation at even higher frequencies than were used in the case of oscillograms *b-f*. The only effect of an interpolated dorsal root volley was slightly to reduce, rather than to augment, the size of the subsequent antidromic volley (record *h*). The reduction can be explained by the refractoriness of some of the motor axons at the stimulating leads, consequent upon their having conducted reflex discharges, and by the collision of centrifugal motor reflex impulses with the centripetal testing impulses.

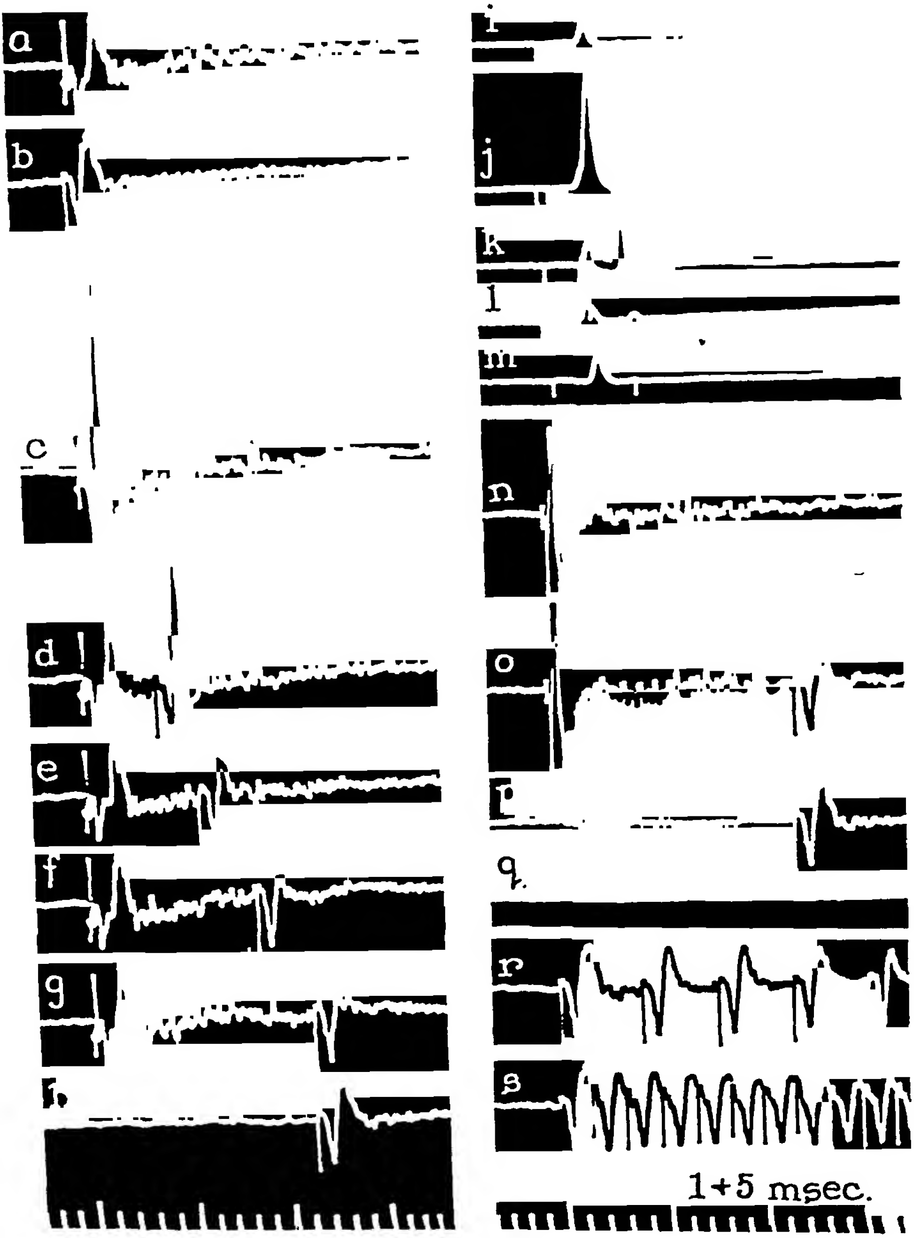

FIG. 3. Excitatory and inhibitory effects of dorsal root volleys on responses of motoneuron somas. Cat under light nembutal, dorsal roots cut. Oscillograms *a-h*, *n-p*, *r* and *s* are micro-electrode records from the L_7 ventral horn. *i-m* and *q* are records from the crural nerve. *a*, L_6 dorsal root volley. *b* and *h*, antidromic volley in crural motor axons. *c-g*, L_6 dorsal root volley conditioning crural antidromic volley. Records *i-m* show reflex discharges set up in crural motor axons by one and two L_6 dorsal root volleys. *n*, L_7 dorsal root volley. *p*, crural antidromic volley. o, L_7 dorsal root volley conditioning crural volley. *q* shows absence of conspicuous reflex discharge into crural axons following L_7 dorsal root volley. *r* and *s*, series of crural antidromic volleys.

The records of Fig. 3 illustrate both facilitatory and inhibitory actions of dorsal root volleys upon the somas of a pool of motoneurons. The preparation was a cat under Nembutal anesthesia. The caudal cord was transected and the ipsilateral dorsal roots cephalad through L_2 were cut. The tested motoneurons were those supplying the muscles of the quadriceps group. The testing centripetal motor volleys were initiated by the application of stimuli to the cut central ends of the nerve branches to the quadriceps. Conditioning volleys were set up in the L_6 and the L_7 dorsal roots. A micro-electrode was inserted into the lateral portion of the ventral horn at an axial level corresponding to the middle of the L_6 segment. This locus is within the pool of motoneurons which supply the muscles of the quadriceps group (10, 7).

The potential changes produced at the micro-electrode reveal that the somas of the tested (quadriceps) motoneurons were affected by the central effects produced by the arrival at the cord of the L_6 dorsal root volleys (records *a-h*). Record *a shows the effect of an* L_6 *dorsal root volley in isolation*; records *b* and *h* the responses to stimulation of the deafferented nerve branches to the quadriceps muscle. When the shock to the L_6 dorsal root and that to the quadriceps nerve were delivered in sequence so that the antidromic volley arrived at the cord shortly after the arrival of the impulses in the dorsal root fibers (record *c*, also *d*), the response of the motoneurons was greatly augmented. When, however, the antidromic volley arrived a few msec. after the dorsal root impulses, the motor impulses failed to enter the somas of the motoneurons to any extent. The inhibition of the response of the somas was nearly complete (records *f* and *g*). The time course of the conditioning of the response of the somas of the motoneurons to the antidromic volley roughly paralleled the conditioning by an L_6 dorsal root volley of the response of the motoneurons to a subsequent L_6 dorsal root volley (records *i-m;* cf. 9).

Careful comparison of records *c-g* with *i-m* of Fig. 3 reveals, however, that the response of the motoneuron somas to antidromic stimulation and the reflex discharges of motoneurons of the same pool were not conditioned in exactly the same time course. The conditioning sensory volley facilitated the direct response of the motoneuron somas to a centripetal motor volley for a longer period than it facilitated the reflex discharge to a dorsal root testing volley. The available data do not permit resolution of the alternative explanations for this difference.

In preparations such as the one under consideration an L_7 dorsal root volley typically fires no quadriceps motoneurons (Fig. 3 *q*) and has only an inhibitory effect on the reflex discharge of these motoneurons to a simultaneous or subsequent L_6 dorsal root volley (9). Correspondingly the response of the quadriceps motoneurons to a centripetal volley in their own axons, as shown in isolation in record *p*, was greatly depressed by a preceding L_7 dorsal root volley (record *o*). It is obvious that in this case the depression of the response of the somas could not have been due to subnormality of

motoneurons, since none were fired by the conditioning afferent volley.

It is apparent that the properties of motoneurons are altered by activity in the cord even when this activity itself does not fire the motoneurons. The degree to which the activity in sensory fibers and premotor neurons determines the state and excitability of the motoneurons is emphasized by noting that the motoneuron soma response deficit produced by an antecedent L_7 dorsal root volley which fired no motoneurons (record *o*) was greater than the deficit produced by direct repetitive activity of the motoneurons at high frequencies (records *r* and *s*).

The conspicuous augmentation of the response of the motoneuron somas has a delay, measured from the time of arrival of the dorsal root impulses, which is comparable with the synaptic delay at motoneurons as determined by Lorente de Nó (3, 4) and Renshaw (8). The illustrative records of Fig. 4 were taken from an experiment on a rabbit under light Nembutal anesthesia. The caudal cord was severed and all the dorsal roots on one side as far cephalad as L_2 were cut intradurally. Stimulating electrodes were placed on the tibial nerve in the thigh and on the combined ipsilateral dorsal rootlets of the L_7 and S_1 segments. Records were taken of the potential changes produced at a micro-electrode placed in the ventral horn. Record *a* shows the response to the dorsal root volley alone, and *b* the response to a tibial antidromic volley in isolation. In both records the time of arrival of the impulses at the ventral horn was approximated by the moment at which the initial positive deflection passed through the baseline to rise into the negative spike. Both the sensory impulses and the antidromic motor volleys were initiated in each of the subsequent oscillograms (*c-g*). Records *c* and *d* are approximately algebraic summations of the two responses in isolation.

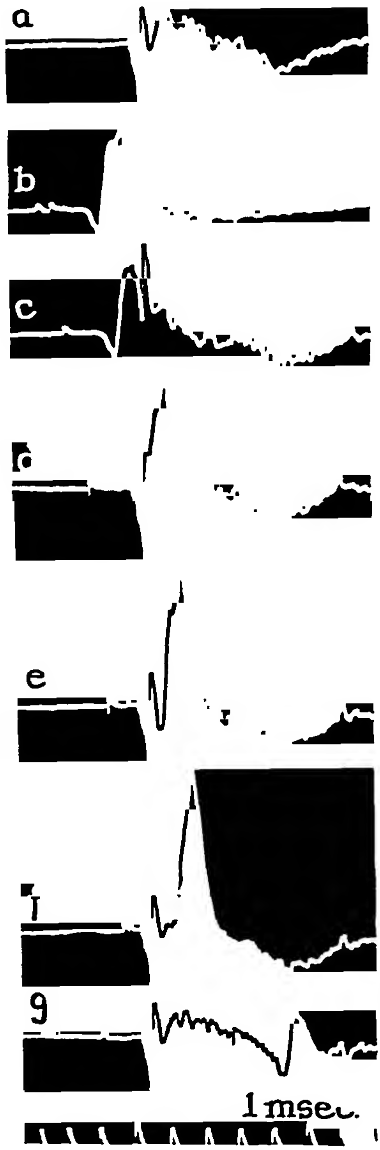

FIG. 4. Relation between time of arrival of dorsal root volley at the spinal cord and its conditioning effect on the somas of motoneurons. Rabbit under light nembutal, dorsal roots cut. Micro-electrode records from the ventral horn. *a*, L_7 plus S_1 dorsal root volley. *b*, tibial antidromic volley. *c-g*, both.

When, however, the dorsal root volley definitely preceded the arrival of the antidromic impulses (records *e* and *f*) it is seen that those portions of the motoneuron negativity which followed the arrival of the dorsal root impulses by 0.9 msec. and a little more were definitely augmented. This interval of 0.9 msec. represents an average value for the duration of synaptic delays at spinal motoneurons as well as for the central reflex times of two-neuron reflex arc discharges (8).

Examination of any of the figures, particularly of Fig. 4, reveals that the augmentation and depression of the electrical response relegated to the motoneuron somas occur *immediately* upon arrival of the antidromic testing volley at the cord. Therefore the part of the so-called motoneuron soma response which is conditioned by sensory impulses could not in reality be due to neurons of the ventral horn which might be excited after a synaptic delay by impulses in the recurrent collaterals of the testing motor axons.

Discussion

A plausible interpretation for the present findings is that impulses are conducted with a decrement at the soma of the motoneuron. The central changes initiated by dorsal root volleys would then augment, or as the case may be decrease, the extent to which antidromic impulses penetrate into the cell body and the dendrites. Consequently an antidromic impulse would travel less far, or further, into the soma of a motoneuron than when unconditioned by a dorsal root volley. This would suggest that, in contrast to conduction of impulses along the normal axon (2, 11), the "factor of safety" for conduction at the soma is not large. Indeed, any factor of safety which may exist can be abolished as a consequence of the central effects initiated by an antecedent dorsal root volley, even by a volley which produces no reflex discharges of the tested motoneurons.

To illustrate the meaning of this conclusion, suppose that a motoneuron is excited by the detonator action of impulses in fibers which terminate on some particular portion of the soma. Whether or not the activity would spread over the soma to excite the axon would depend upon the excitability and responsiveness of the intervening regions of the soma. The state of these intervening regions of the soma would depend upon the excitatory and inhibitory effects exerted by the instantaneous state of activity in the cord, as well as by the previous activity of the soma itself.

This conclusion is based on experiments with cats and rabbits which had been subjected to the following experimental manipulations: (i) they were either decerebrated or lightly anesthetized; (ii) they had undergone a laminectomy; (iii) in each case at least one ventral spinal root had been cut; (iv) a small micro-electrode was necessarily inserted into the ventral horn from which the records were taken. However, it does not seem probable that any of these conditions could alter the properties of the motoneurons in a radical manner.

SUMMARY

A centripetal volley of impulses which arrives at the spinal cord over ventral root fibers of cats and rabbits produces a series of potential changes in the ventral horn. A brief initial positive potential-change signals the approach of the impulses in ventral root axons. A negative deflection of a duration of 1–2 msec. follows. It is due to the invasion of the motoneuron soma (cell body and dendrites) by the centripetal impulses. The negativity passes over into a much more prolonged and smaller positive phase. A rapid series of essentially identical centripetal volleys evokes negative deflections of diminishing size. It is concluded that the response of the motoneuron soma is modified by frequencies of stimulation (*e.g.* 300 per sec.) which the motor axons follow readily. These observations on spinal motoneurons confirm Lorente de Nó's findings for cranial motoneurons.

Conditioning dorsal root volleys alter the motoneuron soma response to centripetal testing volleys in motor axons. The negative potential-change in the ventral horn due to the motoneuron somas is increased, or as the case may be, depressed. The result depends upon the conditioning dorsal root fibers, the tested motoneurons, and the interval between the arrival at the cord of the conditioning and the testing volleys.

It is concluded that retrograde conduction in the motoneurons occurs with a decrement. The degree of penetration into the cell body and dendrites can be augmented or decreased by the consequences of activity in sensory and premotor neurons.

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POSITIONAL NYSTAGMUS IN CEREBELLAR LESIONS

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Introduction

The existence of nystagmus in cerebellar lesions is rather controversial. One group of authors denies a nystagmus of strictly cerebellar origin and is inclined to explain nystagmus in cerebellar diseases as due to accompanying lesions of the vestibular nuclei and fiber systems, *e.g.*, due to edema or pressure in cerebellar tumor or abscess (1, 2, 3, 4, 5); others admit that a lesion limited to the cerebellum may produce at least a transient nystagmus (6, 7, 8) particularly if it affects the deep cerebellar nuclei (9).

The differences of opinion may, in part, be due to differences in the technique of observation. In the majority of the clinical as well as of the experimental studies of cerebellar lesions no account is given whether the influence of the position of the head upon the appearance of spontaneous nystagmus was ascertained although Brun's symptom (vertigo influenced by position of the head) is not infrequent in tumors of the posterior fossa. Otologists (1, 10, 11) seem to have paid more attention to the influence of position upon various types of nystagmus than neurologists, and particularly Nylén showed that positional nystagmus may be observed rather frequently (in 80 per cent) in tumors of the posterior fossa. As pointed out by Nylén himself the nystagmus elicited by changes of position in cases of tumors may be due to changes in pressure or traction affecting the vestibular nuclei, or to changes in the blood supply or of the circulation of the spinal fluid. The question, however, is not answered by this material whether in non space-taking lesions of the cerebellum nystagmus appears more readily in certain positions.

Our attention was directed to this problem in former experiments in which lesions were placed in the posterior part of the vermis (Sven Ingvar's lobus posterior medianus) of cats and preponderantly vertical nystagmus appeared that was increased in abnormal positions, particularly in supine position of the animal (12). The question arose whether positional nystagmus may be elicited from the posterior part of the vermis only or also from other areas of the cerebellum and what mechanism is responsible for this phenomenon.

Methods

The experiments were performed on 39 cats. The lesions were placed in the various parts of the cerebellar cortex by electrocautery and in the cerebellar nuclei by electrolysis with the aid of the Horsley-Clarke stereotaxic apparatus. The animals were operated under ether anesthesia, so that the effect of the anesthetic subsided quickly and the eventual ocular reactions could be observed within half an hour after operation. The eyes were

routinely observed while the animal was tied in normal prone position to the Harvard animal board and the head fixed with the vertex upward in a Czermak head holder which was modified in such a way that the eyes remained free. Thus the relative position of head to trunk remained fixed and the effect of neck reflexes elicited by changes in the tension of the neck muscles was excluded.

The eyes were as a rule examined in the following positions of the animal: normal, right, and left side position, and supine position. The abnormal positions were obtained by slowly tilting the board on which the animal was strapped in order to avoid eventual kinetic labyrinthine reactions. Care was also taken to support the body of the animal sufficiently in the abnormal positions so that the relationship between trunk and head remained unchanged. The extent of the lesions was studied macroscopically and in decisive cases also microscopically on serial sections stained by Weil's method and with cresyl violet.

Results

Localization. Rather small lesions of the lobus posterior medianus may be sufficient to produce positional nystagmus as shown by the following experiment.

Experiment 1. (Cat. 8). Cauterization of the lobus posterior medianus, Oct. 23, 1940, 2–3 p.m. After operation the eyes deviated downward; nystagmus upward, of small amplitude, 36 jerks in 30 sec.

Postoperative notes. First day (Oct. 24). In the *normal position* there was no nystagmus. Between the volitional eye movements there were one to 2 jerks upward appearing per minute. It was difficult to decide whether these jerks were involuntary or not.

Supine position. (Vertex downward). There was nystagmus toward the lower lid.

1st 10 sec.—25 jerks of large amplitude
2d 10 sec.—15 " " " "
3d 10 sec.—14 jerks, amplitude of nystagmus also decreased

In the *normal position* there was no nystagmus. In the *lateral position* (with the left ear downward), there was minimal rotatory nystagmus to the right. With the right ear downward, there was no nystagmus.

Second day (Oct. 25). No nystagmus was present in the *normal position*, but in the *supine position* it was present toward the lower lid.

1st 10 sec.—25 jerks	4th 10 sec.—9 jerks
2d 10 sec.—16 "	5th 10 sec.—5 "
3d 10 sec.—11 "	

Fourth day (Oct. 27). In the *normal position* the animal continued to show no nystagmus. *Supine position.* There was nystagmus toward the lower lid of small amplitude.

1st 10 sec.— 5 jerks
2d 10 sec.— 5 "
3d 10 sec.— 3 "

Fifth day (Oct. 28). In *normal position* no nystagmus was present. In *supine position* 3 trials showed no definite nystagmus. In 1 trial 3 jerks of small amplitude were observed in the 1st 10 sec. only.

Sixth day (Oct. 29). In *normal position* 1–2 upward jerks appeared. In *supine position* 2 trials gave no definite reaction. In 1 trial there were in the 1st 10 sec. 1–2 rotatory↷ jerks and 1 to the upper lid.

Eighth day (Oct. 31). In *normal position* there were 1–2 upward jerks per minute. In supine position (vertex downward) 2 trials gave no definite reaction. In 1 trial 1–2 rotatory ↷jerks were observed. The animal was in excellent condition.

Eleventh day (Nov. 3). In *normal position* there were 1–2 jerks per minute; their direction was ↑ and slightly to the left. In supine position (vertex downward) there was nystagmus toward the lower lid, of very small amplitude.

1st 10 sec.— 4 jerks	4th 10 sec.—0 jerks
2d 10 sec.— 4 "	5th 10 sec.—0 "
3d 10 sec.— 1 "	

On the 25th and 33d postoperative days there was no nystagmus observed in the normal as well as in supine position. The animal was sacrificed on the 33d day (Nov. 25).

Autopsy. Dura adherent to pyramis and uvula. Lesion of these lobules extended to the medial part of the left paramedian lobe (Fig. 1).

Histological Examination. Sections through the beginning of the medulla oblongata reveal lesion of the surface of the *pyramis cerebelli*. On more cranial sections (through the caudal third of the oblongata) in the deeper parts of the pyramis only the central white matter of the pyramis cerebelli is affected and some Purkinje cells on the ventral surface of this lobe are destroyed or show tigrolysis. In these levels the *uvula* is severely injured in all layers except the dorsal surface (Fig. 2 shows demyelination of the medullary layer of its lobules and necrosis of the granular layer). In the *left paramedian lobe* the most ventral lobule shows loss of cells in the granular and Purkinje cell layers on its ventral surface. The *right paramedian lobe* is intact. In the most anterior part of the uvula (sections through the triangular nucleus) the lesion of the uvula decreases in extent and intensity and the *nodulus* shows only slight impairment of the Purkinje cells. The *deep cerebellar nuclei* do not show a definite lesion. There is only slight increase in the number of glial nuclei in the region of the nucleus fastigii. In the Weil preparation one finds on one place a slight disintegration of the myelin sheaths dorso-lateral to the left nucleus fastigii. In the *medulla oblongata* there are only in its most caudal levels (where the central canal is still closed) small foci of necrosis in the nucleus Goll of one side and in the ncleus Burdach of both sides, and one small focus in the lateral part of the left dorsal nucleus of the vagus. The rest of the oblongata and pons including the vestibular nuclei and the posterior longitudinal fasciculi do not show any lesions. In the caudal part of the fourth ventricle necrotic material is found.

FIG. 1. Cat 8.

Summary: Maximal changes in the uvula, slight lesion on the surface of the pyramis cerebelli, and in a lobule of the left paramedian lobe. Very slight injury to the nodulus. No definite lesion of the deep cerebellar nuclei. Vestibular nuclei and their fiber systems intact.

Positional nystagmus may appear, not only following lesions of the lobus posterior medianus, but also after lesion of other parts of the vermis. While, however, rather small lesions of the lobus posterior medianus produced a definite positional nystagmus of several days duration, the nystagmus following lesions of the middle parts of the vermis between sulcus primarius and sulcus prepyramidalis (median part of the lobus simplex and C_2 of

FIG. 2a. and 2b. Cat. 8.
D = Demyelination. G = Granular layer, necrotic.

Bolk, or lobus medius medianus of Sven Ingvar) was much less pronounced and lasted only a few hours after operation.

Experiment 2. (Cat 38). Cauterization of the lobus medius medianus (June 26, 1941) followed by ↑ nystagmus with ↶ component in normal position (frequency 2, 2, 3 in successive 10 sec. intervals) *and nystagmus toward the lower lid with ↶ component or purely ↶ nystagmus in supine position* (frequency 15, 9, 9, in successive 10 sec. intervals).

FIG. 3. Cat. 38.
FIG. 4. Cat. 28.
FIG. 5. Cat 36.
FIG. 6. Cat 27.
FIG. 7. Cat 24. Lateral view of the right cerebellar hemisphere.

Postoperative notes. First day (June 26). Four hours after the operation there was no nystagmus noticeable in any position.

The anatomic examination revealed a lesion reaching from the anterior part of the tuber to the most caudal lobules of the lobus anterior (Fig. 3).

As for the lobus anterior, small lesions affecting only parts of the lobus centralis and culmen were ineffective. Definite positional nystagmus appeared after extensive lesions of these lobules, but outlasted the operation for a few hours only.

Experiment 3 (Cat 38). Cauterization of the lobus centralis and culmen except the most lateral parts on the left side. Feb. 24, 1941, 11–12 a.m. *Transitory positional nystagmus.*

Postoperative notes. First day.(Feb. 24). Half an hour after operation there was no nystagmus in *normal position.* In *right side position* there was nystagmus chiefly to the left canthus, while some jerks were directed toward the upper lid (frequency in successive 10 sec. intervals 18, 18, 14). In *left side position* there was nystagmus of large amplitude to the right canthus (frequency in successive 10 sec. intervals 19, 12, 8). In *supine position* there was nystagmus of smaller amplitude toward the lower lid (frequency in successive 10 sec. intervals 18, 16, 10). Three hours after operation the eyeballs deviated only occasionally

to the right, in normal position of the animal. There were no ocular movements in right or left side position, and in supine position only occasional eye jerks of very small amplitude toward the lower lid.

Anatomic examination: Extensive destruction of the cortex of the lobus centralis and culmen sparing only the most lateral parts on the left side. (Fig. 4)

Still less pronounced were the effects of lesions of the ansiform lobe. In some cases a definite nystagmus failed to appear at all despite extensive lesions of Crus I and II of this lobe (*e.g.*, Cat 36, Fig. 5); in others a weak positional nystagmus appeared immediately following the operation, *e.g.*, in cat 27 cauterization of the right ansiform lobe (Fig. 6); 10 minutes after operation: no definite nystagmus in normal position; ↷ Ny. in left side position; horizontal undulation in right side position; ↶ Ny. in supine position. No nystagmus 3 hours after operation.

As was pointed out by ten Cate (13) it is rather difficult to place strictly isolated lesions of the paraflocculus.* We were, however, able to produce lesions of the paraflocculus with only rather small associated lesions of the Crus I or Crus II of the ansiform lobe. In these cases the appearance of positional nystagmus was somewhat more pronounced than after extensive lesion of the ansiform lobe alone.

Experiment 4. (Cat 24). Cauterization of the dorsal and ventral part of the right paraflocculus. Jan. 17, 1941. 11:30 a.m.–12:20 p.m. *After operation the eyes showed occasional* ↑ *jerks in normal position,* ↷ *nystagmus in left lateral position, no definite nystagmus in supine position and right side position.*

Postoperative notes. First day (Jan. 17). Three hours after operation the animal showed hypermetric movements of the right hind limb when walking. There was no nystagmus in *normal position*. In *left lateral position* occasional ↷ jerks of small amplitude appeared. In *right lateral position* there were slow jerks to the lower lid and somewhat to the right (frequency in successive 10 sec. intervals 4, 4, 2, 3). In *supine position* nystagmic jerks toward the lower lid appeared (frequency in successive 10 sec. intervals 3, 0, 2, 2).

Second day (Jan. 18). Nystagmus could still be demonstrated in right lateral and in supine position, but not in normal and left lateral position. In *right lateral position* it was directed toward the lower lid, sometimes combined with a ↷ component, and had a frequency of 7, 6, 6, jerks in successive 10 second intervals. In *supine position* it was purely ↷ of small amplitude and had a frequency of 4, 5, 6 jerks in successive 10 second intervals.

Anatomic examination: There was a lesion of the dorsal and ventral part of the right paraflocculus encroaching upon the right crus secundum (Fig. 7).

It should be emphasized that positional nystagmus appeared in lesions of the cerebellar vermis, particularly the posterior part, although the medulla oblongata and pons were intact or showed on histological examination of serial sections only small foci of necrosis in the most caudal levels outside the vestibular nuclei and their fiber connections with the eye muscle nuclei (see protocol cat 8).

Since the cerebellum may influence the vestibular nuclei, chiefly by tecto-bulbar systems, special attention was paid to the question whether the lesions must encroach upon the nuclei tecti in order that nystagmus ap-

* In view of the smallness of the flocculus in cats and the concealed position of this structure underneath the paraflocculus on the side of the cerebellar peduncles, cats seem unsuitable for placing isolated lesions of the flocculus, and the influence of this structure upon positional nystagmus will have to be studied in other animals.

pears. In a number of cases positional nystagmus was observed, although the histologic examination failed to reveal definite alterations in the nuclei tecti. This does not exclude, however, the possibility that these nuclei may have been at least temporarily impaired by disturbance of their blood supply. If the positional nystagmus following lesions of the vermis should be caused by a temporary functional impairment of the nuclei tecti, one should expect that direct lesion of these nuclei should produce more pronounced and lasting effects. It seemed, therefore, of interest to compare the effect of lesions of the vermis with that of lesions of the nuclei tecti. These experiments showed that definite positional nystagmus may be produced by injury to these nuclei, but that it is neither more pronounced nor of longer duration than after lesions limited to the vermis (see following protocol).

Experiment 5 (Cat 39). Destruction of the nuclei tecti produced positional nystagmus.

Preoperative notes. 6-30-41. There was no positional nystagmus. Rotation on Bárány chair in normal position (extreme values of several tests):

10 × ↷: Nystagmus to left, 10–21 jerks in 11–12 seconds.
10 × ↶: Nystagmus to right, 15 jerks in 10–11 seconds.

Operation. July 2, 1941. 10:15–11:15 a.m. The nuclei tecti were electrolytically destroyed by a needle which was introduced in horizontal direction in the midline and at 1.5 mm. on either side of the midline 6 and 7 mm. above the calamus scriptorius into a depth of 10 mm.

Postoperative notes. First day (July 2). The animal showed opisthotonus, the forelimbs were in extended position. In *normal position* there was a nystagmus ↓ of small amplitude, 5 jerks in 30 sec. In *supine position* there was a nystagmus of medium amplitude toward the lower lid, sometimes with ↶ component. Frequency in successive 10 second intervals: 10, 10, 11, 15, 16, 16, 16, 23, 13; then the eyeballs rotated toward the lower lid and the nystagmus became undiscernible. In *right side* position there was nystagmus of medium amplitude toward the lower lid. Frequency in successive 10 sec. intervals: 10, 11, 5, 6. In *left side* position there was nystagmus toward the lower lid of small amplitude. Frequency in successive 10 sec. intervals: 8, 6, 6, 5, 5.

Second day. (July 3). Opisthotonus and extended position of the forelimbs were still present. While in *normal position* no nystagmus appeared, the animal showed in *supine position* nystagmus toward the lower lid. Frequency in successive 10 sec. intervals: 11, 10, 9, 9. 14, 15. In *left side position* nystagmus ↷ and toward the lower lid, 5 jerks in 30 sec. In *right side position* there was nystagmus toward the lower lid. Frequency in successive 10 second intervals: 5, 6, 8, 8. Rotation on Bárány chair in normal position. (Extreme values of several tests):

10 ↷: Nystagmus to left, 29–32 jerks in 15–19 seconds.
10 ↶: Nystagmus to right, 25–30 jerks in 13–15 seconds.

Fourth day. (July 5). Opisthotonus was still present, but no nystagmus could be detected in *normal and left side position.* In *supine position* there was nystagmus toward the lower lid of medium, then of small amplitude. Frequency in successive 10 sec. intervals: 0, 0, ?, 3, 3, 5, 4, 4, 2, 3. In *right side position* there was nystagmus of small amplitude to the lower lid, sometimes . Frequency in successive 10 sec. intervals: 1, 3, 2, 3, 2.

Eighth day (July 9). While no definite opisthotonus could be found, the forelimbs were still held in somewhat extended position when the animal walked. There was no nystagmus in *normal* and *right side* position, but occasional jerks could be noticed in *supine position,* (2, 1, 2 in successive 30 sec. intervals, toward upper lid and ↷) and *in left side position* (1, 0, 0, jerks in 30 sec. intervals in ↶ direction). Rotation on Bárány chair in normal position (Extreme values of several tests):

10 ↷: Nystagmus to left, 18–24 jerks in 10–12 seconds.
10 ↶: Nystagmus to right, 16–18 jerks in 8–12 seconds.

Tenth day (July 11). There were only occasional eye movements (0–3 per 30 sec.) in

all positions of the animal. Rotation on Bárány chair in normal position. (Extreme values of several tests).

10↷: Nystagmus to left, 15–17 jerks in 10 seconds.
10↶: Nystagmus to right, 11–15 jerks in 8–9 seconds.

Eleventh day (July 12). There was no nystagmus in any position. The animal was sacrificed.

Histologic examination: Small puncture canals pass through pyramis and uvula; they end in electrolytic lesions destroying the nuclei tecti (Fig. 8) and the right nucleus globosus and encroach upon the left nucleus globosus.

Control experiments showed that normal or anesthetized cats do not show positional nystagmus. Immediately after awakening from ether anesthesia, there may exist a weak spontaneous nystagmus or undulation in

FIG. 8. Cat 39.

normal position. Change of position does not increase this nystagmus, or at most occasionally a few jerks appear immediately after the animal has been turned into an abnormal position (*e.g.*, lateral position).

Characteristics of the positional nystagmus. The positional nystagmus is usually most marked in supine position of the animal, occasionally it is more pronounced in side position, (*e.g.*, sometimes after parafloccular lesions or asymmetric lesion of the vermis or of the nuclei tecti). Its appearance is often preceded by a latent period of a few seconds after the animal has been turned from the normal into the abnormal position. Sometimes, however, jerks appear immediately after the new position has been obtained.

The change from the normal into the abnormal position may not only increase frequency and amplitude of the nystagmus but also influence its

direction. In *normal* position one usually observes in lesions of the vermis, if reactions of the eyeballs appear at all, a downward deviation of the eyeballs and/or a preponderantly vertical nystagmus of low frequency with the fast component to the upper or lower lid as previously described. After the animal has been brought into the *supine position*, there usually appears a nystagmus toward the lower lid of higher frequency and sometimes also larger amplitude than in the normal position. Thus nystagmus toward the upper lid in normal position may be reversed in its relation to the orbit. Combination with a deviation of the eyeballs chiefly towards the lower lid and/or with a rotary component of the nystagmus on one or both eyes could be observed in normal as well as in supine position. In *side position* the nystagmus usually beats parallel to the palpebral fissure or somewhat obliquely, with the fast component to the upper ear. Or rotary nystagmus is observed, *e.g.*, ↷ in left side position and ↶ when the animal lies on the right side.

If one compares successive 10-second intervals, frequency and often also amplitude of the nystagmus diminish rather rapidly after the animal has been placed into the abnormal position. Repeated testing (turning from the normal into abnormal positions) without pause sometimes also produces exhaustion of the reaction so that it may be more pronounced on the first examination than on the subsequent ones.

It seems particularly noteworthy that in a number of cases nystagmus appeared only in abnormal position of the animal (head), while it was absent in the normal position. This could particularly be observed when small lesions had been placed (see Cat 8).

In all experiments the positional nystagmus was only transient and disappeared within about a week following operation. As was already mentioned, this was found not only following lesions limited to the cerebellar cortex and adjacent white matter, but also in lesions of the nuclei tecti (see protocol Cat 39).

Influence of various receptors. As pointed out in the description of the method of examination, neck reflexes were avoided by keeping the head and body of the animal in fixed relative position on the animal board. In a number of experiments retinal impulses were eliminated by covering the anesthetized cornea with a small rubber membrane that was blackened with India ink. This had no effect upon the positional nystagmus. Finally bilateral labyrinthectomy was performed in animals which showed typical positional nystagmus following lesions of the median part of the posterior lobe, as shown by the following protocol.

Experiment 6 (Cat 18). Destruction of uvula and lesion of ventral part of pyramis cerebelli followed by positional nystagmus. After additional bilateral labyrinthectomy only weak spontaneous nystagmus, practically not influenced by changes in position.

First operation (Dec. 9, 1940. 10:40–11:20 a.m.). The uvula cerebelli was mechanically extirpated under ether anesthesia.

Postoperative notes: First day (Dec. 9). At 3:00 p.m. there was tendency to opisthotonus and increased extensor tone of the limbs. In *normal position* a nystagmus of small amplitude

↑ and ↶ was observed (7–9 jerks in 30 sec.). In *supine position* there was nystagmus toward the lower lid and slightly ↶ (31–37 jerks in 30 sec.). In *right side position* there was nystagmus ↶ of very small amplitude (35–36 jerks in 30 sec.). In *left side position* there was nystagmus of small amplitude toward the lower lid (25–27 jerks in 30 sec.).

Second operation 3:45–4:15 p.m. Bilateral labyrinthectomy.

Postoperative notes: First day (Dec. 9) 4:50 p.m. In *normal position* the eyes showed slow vertical ↓ movements, some with ↶ component, occasionally with ← component to the right. In *supine position* there were eye jerks toward the lower lid, some with component to right, 4–6 in 30 sec. In *right side position* there were 5 ↶ jerks to the right in 30 sec. In *left side* position there were 1–4 ↓ and ↶ jerks in 30 sec. Rotation on a Bárány chair: 10 × to the right, then 10 × to the left did not influence the spontaneous ↓ jerks.

Second day (Dec. 10). In *normal position* the eyes showed ↓ and ↶ jerks, 3–8 in 30 sec. In *supine position* there were eye jerks toward the lower lid and ↶; 4–7 jerks in 30 sec. In *left side position* there were slight ↶ eye jerks, 1–4 in 30 sec. In *right side position* there were also ↶ jerks, 1–5 in 30 sec. Rotation on Bárány chair, 10 × to the right, then to left did not influence the spontaneous ↓ and ↶ jerks.

Animal sacrificed.

Anatomic examination: Destruction of the uvula and lesion of the ventral part of the pyramis cerebelli. The inner ear is replaced on both sides by a large cavity that communicates with the internal auditory meatus.

The absence of excitable receptors in the labyrinth was shown by the ineffectiveness of rotation on a Bárány chair. Despite the elimination of the labyrinths a weak spontaneous nystagmus (a few jerks per min.) could still be observed; this was apparently of central origin as will be pointed out later. Changes of position did not definitely influence this nystagmus beyond the variations that it showed spontaneously in normal position.

Discussion

Little is known regarding the influence of the cerebellum upon vestibular reflex arcs in oblongata and pons. Lesion of the vermis-nuclei tecti system may produce, at least in some species, transient overexcitability of these reflex arcs (increase of duration and intensity of experimental nystagmus observed by Bauer and Leidler (14) in rabbits; increase of tonic labyrinthine reflexes upon limb and trunk muscles described by Pollock and Davis (15) in decerebrate cats; negative results reported by Dow (16) in monkeys and chimpanzees after subtotal ablation of uvula and nodulus).

In this group of symptoms of overexcitability of vestibular reflex arcs following cerebellar lesions one may perhaps also include the observations reported in this paper. Such an interpretation is supported by experiments in which we tested not only positional nystagmus but also experimental nystagmus produced by rotation on a Bárány chair before and after the cerebellar operation.

In Fig. 9 the results of such examinations of the excitability of the labyrinth are charted for Cat 39 (lesion of the nuclei tecti, see experiment 5). As an indication of the intensity of the positional nystagmus the number of jerks per 30 sec. in supine and in right side position was chosen. One may notice that the curves of the positional nystagmus and of the postrotatory nystagmus reach their maximum on the same day; although their descend-

ing course is not strictly parallel, they reach the normal level at the same time.

If these experiments are surveyed from the point of view of localization, the phenomenon of positional nystagmus is most marked after lesions of the lobus posterior medianus but may also appear, although less marked and of still shorter duration, after lesions of other parts of the cerebellum, particularly palaeo-cerebellar areas.

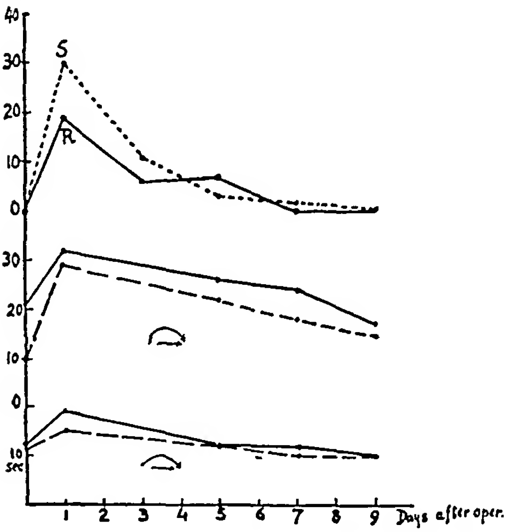

FIG. 9. Experimental postrotatory and positional nystagmus before and after lesion of the nuclei tecti. (Cat 39).
Nystagmus to the left after 10 rotations to the right;
Minimal number of jerks...... — — —
Maximal number of jerks...... ———
Minimal duration............ — — —
Maximal duration............ ———
Position nystagmus in supine position (S ·······) and in right side position (R ———).
Number of jerks in 30 sec.

The question can only partly be answered why the appearance of positional nystagmus depends on the lesion of certain cerebellar areas. One should expect that such an effect is observed after extirpation of such parts of the cerebellum as send efferent fibers to the vestibular nuclei. Thus one can easily understand that lesions of areas which send no or only scanty impulses to these nuclei such as the ansiform lobe are followed by a positional nystagmus of a few hours duration only. The same may apply to the lobus medius medianus whose connections with the nuclei tecti seem less developed than those of the other parts of the vermis (see fig. 93 in Fulton, 17). The definite effect of lesions of the lobus posterior medianus may be explained by the fact that this lobe sends efferent impulses to the vestibular nuclei not only by way of the tectobulbar system but also by way of direct fibers to the vestibular nuclei (from the uvula and nodulus, 18). As for the paraflocculus, Clarke and Horsley (19) described efferent connections with the nuclei tecti, while Dow traced degenerating fibers to the dentate nucleus and nucleus interpositus.

It may seem surprising that extensive lesions of the lobus centralis and culmen of the anterior lobe* produce a positional nystagmus of much shorter duration than lesions of the posterior lobe, although these parts of the anterior lobe send also efferent fibers to the nuclei tecti. Various factors may contribute to this difference. First the possibility should be borne in mind

* The anterior lobe will be discussed here with exception of the lingula, to which a special study shall be devoted.

that lesions of the posterior lobe may more easily affect the nuclei tecti by interfering with their vascular supply than do lesions of the anterior lobe. In view of the fact that direct injuries to the nuclei tecti do not produce a more pronounced and longer lasting positional nystagmus than lesions of the vermis, this factor should not be overestimated.

Second, the lesion of direct efferent fibers from the cortex of the vermis to the vestibular nuclei may play a more important role than the lesion of the cortico-tectal connections. Such direct cortico-vestibular fibers were chiefly traced from the lobus posterior medianus as previously mentioned. According to Saito (20), however, all parts of the vermis, and also the lateral lobes of the cerebellum send direct efferent fibers to the region of the Deiter's nucleus by way of the fibrae perforantes.

Third, the possibility should be considered that the cerebellofugal systems to the rhombencephalon may be functionally differentiated in that certain parts may act chiefly upon the postural reflex arcs and others chiefly upon the vestibulo-ocular reflex arcs. This may apply to the direct cerebello-bulbar fibers (*e.g.*, fibrae perforantes) as well as to the fastigio-bulbar systems. There are indications that the part of these systems originating from the lobus centralis and culmen influences the postural centers of the limb and neck muscles in oblongata and pons. Sherrington (21) and others showed that the extensor rigidity in the limbs of decerebrate cats may be inhibited by stimulation of the anterior lobe only; this reaction can be maintained by cerebello-bulbar systems alone, since it persists after transverse section behind the midbrain (22, 23). In agreement with the stimulation experiments, lesions of lobus centralis and culmen produced only a release of the neck extensors and of the antigravity muscles of the limbs (24). Thus it seems understandable that lesion of these areas have only a slight effect in producing positional nystagmus.

It may be of interest to compare our results with the present trend of cerebellar physiology. A sharp localization of single muscle groups within the various lobules of the cerebellar cortex, as suggested by Bolk and by Rynberk, is not supported by recent stimulation experiments of Hare, Magoun and Ranson (25). This does not exclude, however, a functional localization as proposed by Fulton (17). Our observations seem to agree with such a scheme of localization with the restriction that a functional group such as the vestibular nuclei is influenced chiefly but not exclusively by a certain area of the cerebellar cortex.

The question may be raised whether the influence of changes in the position of the head upon the spontaneous nystagmus, particularly upon its direction, is due to reflex changes in the state of tonic contraction of certain eye muscles produced by tonic labyrinthine reflexes upon these muscles. The nystagmus could of course show a change of direction if it were superimposed upon tonic reflex reactions of the eye muscles. Tonic labyrinthine reflexes upon the eyes play, however, only a minor role in animals with frontal position of the eyeballs in which the visual fields overlap, and the

position of the eyeballs chiefly depends on retinal reflexes (26). In our cats with cerebellar lesions a deviation of the eyeballs to the lower lids was sometimes more pronounced in supine than in normal position; positional nystagmus, however, appeared with as well as without tonic deviation of the eyeballs.

Thus changes in the state of contraction of eye muscles due to labyrinthine reflexes upon these muscles played at the most an accessory part in the mechanism of the positional nystagmus after cerebellar lesions. Static impulses from the labyrinth could, however, act in the center upon the kinetic part of the vestibulo-ocular reflex arc. Lorente de Nó (27) observed on otherwise normal rabbits that a nystagmus produced by rotation or by caloric stimuli may be modified as to intensity, and sometimes also as to direction, by changes in the position of the head although the same stimuli were produced in the semicircular canals. There occurred in these cases apparently an interaction of impulses from the static receptors with those from the semicircular canals. In our experiments we have to do with a nystagmus that persisted, at least to a certain extent, after bilateral labyrinthectomy and was apparently due to an abnormal discharge of the vestibular nuclei that were in a state of increased excitability after the cerebellar operation. One may perhaps assume that in this state of overexcitability there occurs not only an increase of discharges from the vestibular nuclei to the eye muscles resulting in "spontaneous nystagmus," but also a spread of impulses originating in the static receptors of the labyrinth to those parts of the vestibular nuclei which transmit kinetic reactions to the eye muscles. Due to such a spread, impulses from the static receptors may modify intensity and also direction of a "spontaneous nystagmus" or may even cause its appearance in certain positions of the head.

In agreement with such an explanation our experiments showed that changes in the position of the head were no longer able to influence the spontaneous nystagmus after the labyrinths had been eliminated. The fact that a weak "spontaneous nystagmus" may persist for some time after bilateral labyrinthectomy may seem astonishing. The appearance of Bechterew's compensatory nystagmus, when some days after extirpation of the first labyrinth, the second labyrinth is also eliminated, and the observation of nystagmus following punctures of the vestibular nuclei in labyrinthectomized dogs (28) show, however, that the vestibular nuclei are still able to discharge after elimination of the peripheral receptors.

The experience was mentioned that in some of our animals nystagmus was present in abnormal positions only. This seems to suggest that the examination of patients with suspected cerebellar lesions should routinely include an examination of positional nystagmus. Such an examination may yield positive results in certain positions despite the absence of spontaneous nystagmus in normal position.

Summary

After lesion of parts of the cerebellum of cats, particularly of the lobus posterior medianus, nystagmus was observed in abnormal positions of the head, particularly in supine position with the vertex downward. A weak nystagmus in normal position of the head was increased, sometimes also its direction was changed, in abnormal positions; or nystagmus appeared in abnormal positions only. This phenomenon is transient. Electrolytic lesions of nuclei tecti had a similar effect. In analyzing the receptor mechanism, neck reflexes and retinal reflexes could be excluded. Bilateral labyrinthectomy abolished this effect of position, while a weak spontaneous nystagmus could persist. It is assumed that the appearance of positional nystagmus following cerebellar lesions is a phenomenon of release of parts of the vestibulo-ocular reflex arcs, since it is associated with increase of the experimental postrotatory nystagmus. The experiments suggest that the existence of positional nystagmus should routinely be tested in suspected cerebellar lesions.

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EFFECT OF ALKALOSIS AND ACIDOSIS ON CORTICAL ELECTRICAL ACTIVITY AND BLOOD FLOW*

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A DECREASE in CO_2 tension of the blood causes a decrease in frequency and an increase in amplitude of cortical potentials while an increase in CO_2 tension has the opposite effect (2, 6, 9). Whether these changes are directly a function of the CO_2 or are related to variations in the hydrogen ion concentration of the blood has not been clearly demonstrated. The common use of the terms "alkalosis" and "acidosis" are tacit acknowledgement that they are related to the latter. Davis and Wallace (3) have shown that the acidosis or alkalosis produced by oral ingestion of sodium bicarbonate and ammonium chloride respectively cause no significant change in the effect of hyperventilation on the electroencephalogram of man. They suggest that hyperventilation causes the appearance of delta waves by inducing cerebral vasoconstriction, thereby diminishing the supply of oxygen and glucose to the cortex.

This study is an attempt to determine (i) the effect of alkalosis and acidosis produced by the intravenous injection of chemicals on the cortical electrical activity of the anesthetized cat; (ii) the relationship of the respiratory changes in alkalosis and acidosis to the electrocorticographic changes; and (iii) the role of cerebral blood flow in the production of changes in cortical potentials due to alkalosis and acidosis. This was attempted by means of the simultaneous recording of cortical potentials and the measurement of pial vessel size through a skull window.

METHODS

The cats were firmly fixed on a heated animal holder after being anesthetized with pentobarbital sodium (approximately 0.03 g. per kg. of body weight intraperitoneally). A Forbes' lucite window was screwed into a threaded trephine opening after exposing an area of cortex in the anterior portion of the parietal lobe. A silver-tipped screw was inserted into one of the openings in the window, thus serving both as a monopolar electrode and a seal for preventing the escape of cerebrospinal fluid. The indifferent electrode was placed on the ear. Electrical activity was recorded with electro-dynamic ink writers driven by a resistance-capacity coupled amplifier (time constant = 0.25 sec.). The input stage of the amplifier was of the differential type thus facilitating recording with a minimum of shielding.‡ Photographs of the pial vessels were taken through the skull window at frequent intervals with the use of a Leitz Ultropak microscope and a reflex camera, using 35 mm. panchromatic film. The negatives were subsequently enlarged and measurements made of arteries ranging in size from 50 to 250 micra in diameter.

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‡ Mr. C. W. P. Walter designed and constructed the recording apparatus.

After preliminary trials with other chemicals and various concentrations, a 0.1 N solution of HCl and a 2.0 N solution of Na_2CO_3 were found to be the most suitable means of producing characteristic changes in the respiratory rate with a minimum of toxic effects. This work is based on 21 injections of HCl and 25 injections of Na_2CO_3 in 12 animals. Because of the buffering capacity of the blood, the rate of injection was an important factor; however the change in respiratory rate has been found to be an index of pH change following injections of acids or alkalis (7). The average amount of HCl injected was approximately 0.0001 moles per kg. of body weight and that of Na_2CO_3 was approximately 0.001 moles per kg. of body weight.

The electrical activity recorded from the cortex of the cat under pentobarbital sodium anesthesia consists of a series of high voltage bursts at a frequency of about 7 to 10 per sec. with little activity between the bursts. This is similar to that seen in dial anesthesia and probably affords the most reliable base line for experimental studies (1).

Results

a. *Acid injection:* Injections of amounts of HCl necessary to produce a definite increase in the respiratory rate in no case caused any definite change in cortical potentials (Fig. 1A). In most cases, moreover, when larger, near-fatal amounts were injected the only change noted was a disappearance of all activity. Occasionally, however, with these large amounts of acid, low voltage, fast activity (11–16 per sec.) was produced and the slower (7–10 per sec.) activity seen in bursts in normal anesthetized animals was ob-

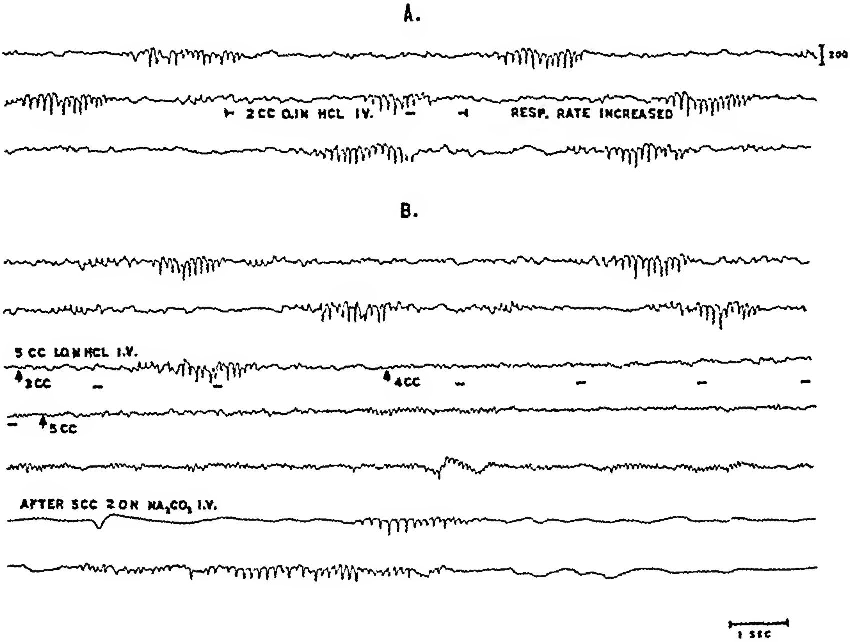

FIG. 1. A. No change is noted in the cortical potentials after injection of amounts of HCl that produce a definite increase in the respiratory rate. B. Injection of a near-fatal amount of HCl is followed by a train of rapid waves of low voltage. Subsequent injection of Na_2CO_3 results in the disappearance of these waves. After an interval in which no activity is recorded, the large amplitude waves seen in the normal record return.

literated (Fig. 1B). This rapid activity was noted, however, only when it was discernible to some extent in the record preceding the injections.

b. *Alkali injection:* Amounts of Na_2CO_3 necessary to produce a definite decrease in the respiratory rate and sometimes twitching and muscular hyperirritability ordinarily caused no definite change in cortical potentials (Fig. 2A and 3A). Occasionally, and even here usually with doses larger than required to produce respiratory changes, there was a transient obliteration

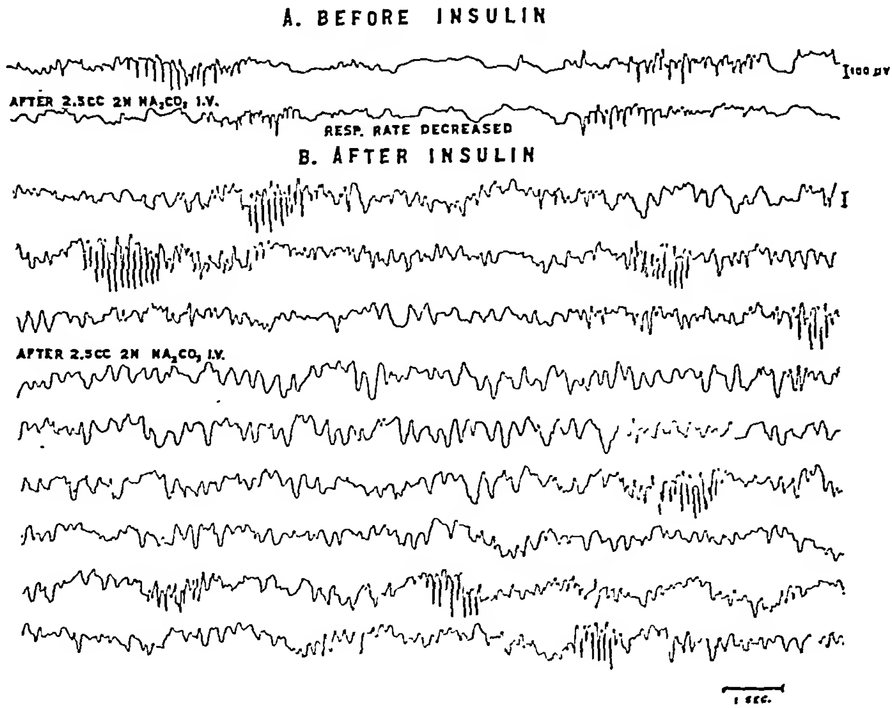

FIG. 2. A. Injection of amounts of Na_2CO_3 sufficient to produce a definite increase in the respiratory rate cause no remarkable change in cortical activity. B. About 90 minutes following the injection of 40 units of insulin many slow waves have made their appearance. After the injection of the same amount of Na_2CO_3 as in A there is a marked increase in the number of slow, high voltage waves (similar to those in Fig. 4A).

of all activity, possibly associated with anoxia. In one animal, however, in which much slow random activity was present (possibly due to trauma) injection of Na_2CO_3 in amounts that ordinarily produced no change, resulted in a series of smooth, high voltage 4–6 per sec. waves, similar to those sometimes seen after hyperventilation (Fig. 4), The injection was repeated four times with the same result.

This suggested the employment of insulin hypoglycemia as a means of changing the normal record and then injecting alkali. Preliminary injections at first produced no change in cortical activity (Fig. 2A and 3B). In the animal whose records are illustrated in Fig. 2 considerable slow activity,

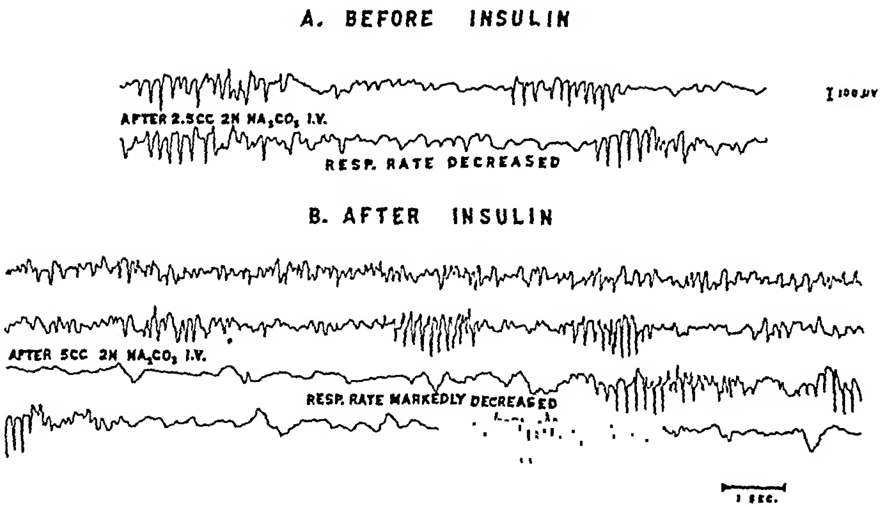

FIG. 3. A. Following the injection of Na_2CO_3 there is a decrease in respiratory rate with no change in cortical activity. B. One hour after the injection of 40 units of insulin Na_2CO_3 injection is followed by the disappearance of all activity except a few slow waves. The large amplitude bursts return before the smaller amplitude rapid activity which was recorded prior to the administration of Na_2CO_3.

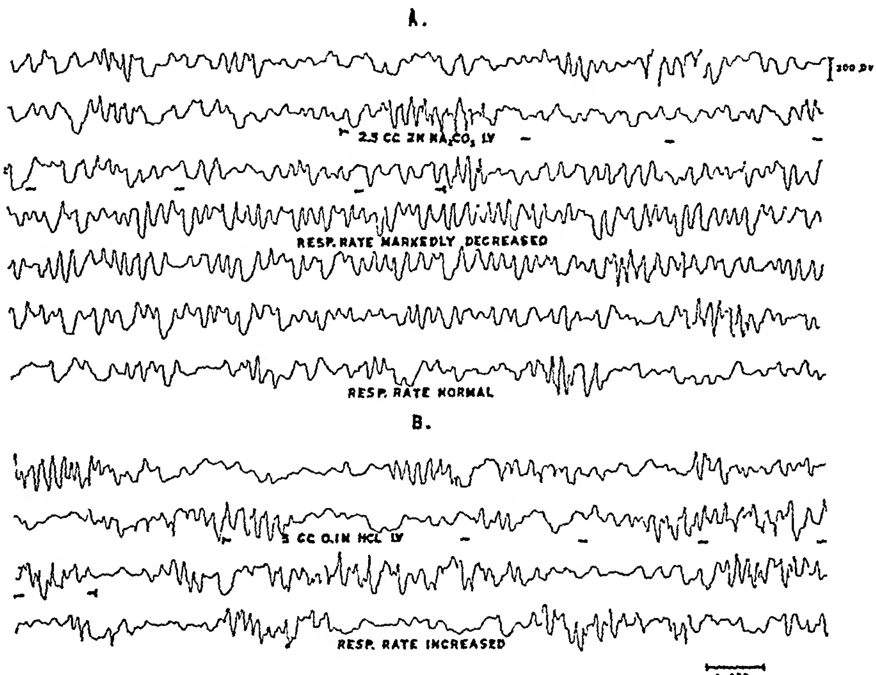

FIG. 4. A. Slow random activity and none of the ordinary bursts are present. Administration of Na_2CO_3 results in an increase in high voltage, four to six per second waves. B. Injection of HCl causes no remarkable change

some having a voltage of about 100–150μV, was present in addition to the usual bursts about 90 min. following the injection of 40 units of insulin. The injection of Na_2CO_3 in the same amounts as in the control period resulted in a series of rather regular, high voltage slow waves, usually four per second, (Fig. 2B), which were similar to those observed after the injection of Na_2CO_3 illustrated in Fig. 4A. Return of the electrical activity to a prealkalotic level occurred in about the same time in both instances. In another animal (Fig. 3) considerable fast activity of lower voltage was present about 60 minutes after the injection of insulin. Na_2CO_3 alkalosis caused all activity to disappear; the slower, high voltage bursts returned long before the faster waves,

Table 1. Effect of injections of hydrochloric acid and sodium carbonate solutions on the diameter of pial arteries

	Constriction					Per cent	Dilatation							Total		
	per cent 25	20	15	10	5	0	per cent 5	10	15	20	25	30	35	Constriction	No Change	Dilatation
HCl	0	0	0	0	0	5	4	4	2	3	0	0	2	0	5	15
Na_2CO_3	1	1	4	12	12	12	7	2	0	0	0	0	0	30	12	9

their frequency (8 per sec.) about the same but slightly larger in amplitude Similar obliteration of fast activity by Na_2CO_3 is seen in Fig. 1B.

c. *Pial vessel size:* Twenty measurements of the diameter of pial arteries were made in 6 animals following 9 injections of hydrochloric acid solution. Fifteen of these measurements revealed vasodilatation ranging from 5 to 35 per cent. Five measurements revealed no change in vessel size; in four of these five the minimal amount of acid injected was 1 cc. of a 0.1*N* solution. In no case was vasoconstriction observed.

Fifty-one measurements of pial artery size were made in 9 animals following 22 injections of a solution of sodium carbonate. Vasoconstriction, from 5 to 25 per cent, was noted in thirty of these measurements; the constriction was 10 per cent or less in twenty-four. No change in vessel diameter was noted in twelve; vasodilatation of 5 to 10 per cent was noted in nine.

Vasodilatation following the injection of other acids and vasoconstriction following injection of other bases have frequently been observed (13). According to Poiseuille's Law the rate of flow is proportional to the fourth power of the diameter of a tube. If this law is applicable to cerebral blood flow (as it seems to be elsewhere in the body, 5) these relatively small changes in diameter represent marked changes in blood flow, as illustrated in the table at the top of the next page.

These changes in vessel diameter which probably represent significant changes in blood flow occurred without relationship to variations in the cortical potentials simultaneously recorded. In fact, the slow waves shown in Fig. 2B following injection of sodium carbonate were accompanied by a

Table 2. Change in blood flow according to Poiseuille's law.

Per cent change in diameter	Flow (100 = Initial flow)	
	vasodilatation	vasoconstriction
5	122	81
10	146	66
15	175	52
20	207	41
25	244	32
30	286	24
35	332	18

slight increase in diameter of the pial vessels. This does not tend to support the suggestion of Davis and Wallace that slow waves following hyperventilation are due to vasoconstriction and cerebral anemia.

Discussion

It seems clear, at least in the case of the agents used here, that there is no direct relationship between the respiratory rate and the frequency of cortical potentials in normal anesthetized animals. Marked respiratory changes may be present with no appreciable alteration in the electrocorticogram. When alterations in frequency do occur, however, they tend to occur in the same direction as the respiratory changes, as suggested by Gibbs and Gibbs (8); that is, a decrease with alkalosis and an increase with acidosis. The cortex seems to be more stable in the presence of changes of pH than does the respiratory center. The chemo-receptors in the carotid body (10) may contribute to this difference, there being no evidence at present for any such accessory mechanism in the case of cortical activity (9).

Normal cortical activity in the cat is influenced only by extremely large, near-fatal amounts of acid or base, as far as can be determined from these records. This would suggest that changes produced by increasing or decreasing the CO_2 tension of the blood are related to the effect of CO_2 itself rather than the resultant change in hydrogen ion concentration of either the blood or tissues. The same has been suggested for the respiratory center; Hooker, Wilson and Connett (11) state that while alkalosis tends to depress the respiratory center and acidosis to stimulate it, blood containing a high tension of carbon dioxide causes greater activity of the respiratory center than another specimen of the same hydrogen ion concentration but with a low carbon dioxide tension. Perhaps it would be preferable to drop the use of "alkalosis" and "acidosis" when referring to such changes.

On the other hand Marshall and Nims (12) assert that the pH at the surface of the cortex does not parallel that of arterial blood following intravenous injection of fixed acids or bases and that hydrochloric acid results only in a momentary period of acidity of the cortex and that sodium bicarbonate produces inconsistent changes in the cortical pH. Gesell and Hertz-

man (7) find this to be true in regard to sodium bicarbonate; they state that increased respiration (as seen in acidosis) commonly occurs following its intravenous administration. However, they express the belief that sodium carbonate (used in our experiments) constantly produces a more alkaline state in the tissues and believe that the respiratory rate is a good index of pH change in the tissues following administration of acids or bases.

In spite of the stability of the electrocorticogram in regard to change in hydrogen ion concentration of the blood, alterations in pattern do occur in the presence of large changes or under additional abnormal conditions, as in insulin hypoglycemia. When they do occur, they appear to depend to a certain extent on the pattern present prior to the injection. "Smooth" waves in general remain smooth (Fig. 4A) and "sharp" waves remain sharp (Fig. 3B). An excess of base tends to mobilize high voltage slow activity and to obliterate low voltage fast activity while acid tends to produce the reverse effect. Dubner and Gerard (4) have described an increase in frequency from the lateral geniculate body following an increase in hydrogen ion concentration, but state that alkalinity (produced by NaOH) abolishes all electrical activity from this region.

Summary

1. The minimal amount of acid (HCl) or alkali (Na_2CO_3) injected intravenously necessary to produce changes in the respiratory rate of the anesthetized cat does not disrupt the cortical potentials.

2. Changes in the electrical activity of the cortex following acidosis or alkalosis occur only if near-fatal amounts are injected or, in the case of alkalosis, if an abnormal pattern has already been established as in insulin hypoglycemia.

3. While intravenous injection of acid causes dilatation of the pial arteries in most cases and intravenous injection of alkali causes constriction in most cases, these changes are not obviously related to the alterations of cortical potentials.

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A DEFICIENCY IN THE PHRENIC RESPIRATORY DISCHARGES PARALLEL TO RETROGRADE DEGENERATION

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THE ANATOMICAL CHANGE occurring in nerve cells after the section of their axons has been extensively studied in the pursuit of neuroanatomical knowledge by the method of Nissl. Little effort has been made, however, to correlate these changes with function. The present study was designed to test whether, in the course of retrograde degeneration, the physiological properties of neurons are altered. As a first approach to the subject, the phrenic was studied.

METHOD

Cats of either sex, weighing from 2 to 5 kg. were used. Under ether anesthesia either phrenic nerve was cut aseptically with a minimum of traction. Two to 200 days later the animal was anesthetized with dial (Ciba, 0.7 cc. per kg. intraperitoneally), a tracheal cannula was inserted, and the phrenics were exposed in the neck. One or more of the roots of each nerve were carefully dissected for a length of 2 to 3 cm. and cut distally. The nearby muscles were retracted and the space so exposed was made into a moist chamber by pads of cotton wet with mammalian Ringer's solution. The distal end of each root was crushed and the nerve was suspended in the air upon a pair of silver wire electrodes (interelectrodal distance 6 to 15 mm.), with the crushed area on the distal electrode for monophasic recording. The region of the nerve from which the record was taken was always at least 10 mm. from the neuroma which resulted from previous section. At this level the two nerves were grossly indistinguishable to the eye.

The respiratory discharges were led through a capacity coupled amplifier to a cathode-ray oscillograph. The coupling condensers were arranged so as to minimize slow shifts in the record and emphasize signals with the time characteristics of axon spikes. The amplified discharges were also passed through a loud speaker. In some instances acoustic analysis was more informative than the photographic record of the cathode ray.

The respiratory discharges in symmetrical roots of the two phrenics were compared. In order to avoid artificial differences in the amplitude of the recorded signal in each experiment the two roots were prepared, as nearly as possible, in the same way, and the conditions of recording were kept constant. Significant differences (greater than 20 per cent) in the amplitude of the records from the two phrenics were found in 3 of the 12 control animals. Hence the results to be presented must be considered quasi-statistically.

At the conclusion of the experiments some of the animals were perfused with chloral hydrate and formamide as recommended by Bank (1). Segments 4, 5 and 6 of the cervical cord were then removed and one of them stained for synaptic end-bulbs by the Davenport (3) modification of Cajal's silver method. Other segments were serially sectioned at 10 to 12μ and alternate sections stained by the Nissl and Bodian methods. The fixative used allowed excellent preparations to be made with all three methods.

RESULTS

In the animals tested 2 to 7 days after section of one phrenic nerve the spontaneous respiratory discharges from the two phrenic nerves were not significantly different. Those examined from the 8th to the 21st day showed a progressive decrease of the amplitude of the discharge recorded from the

previously sectioned phrenic. By the end of the second week this discharge was only about a third of that from the control side (Fig. 1). A week later the change was most marked; in 2 of 3 animals tested after this interval no respiratory discharge could be distinguished from the background noise on the side of the operation, whereas the control phrenic produced discharges of the usual size. After 21 days there was a gradual recovery of the discharge from the previously sectioned nerve, which reached the magnitude of the control between the 40th and 78th days.

The time course of this decreased respiratory discharge following section of one phrenic is charted in Fig. 2. Each rectangle on the graph represents one experiment. The amplitude of the respiratory discharges recorded from the previously sectioned phrenic, expressed as per cent of that of the acutely sectioned, control phrenic, is plotted against days elapsed since the test

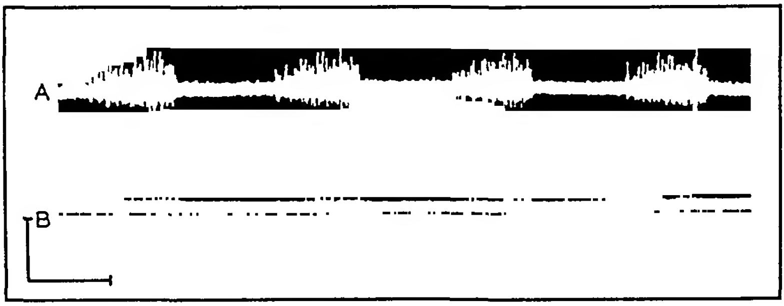

FIG. 1. Deficiency of respiratory discharge recorded from the right phrenic nerve, which had been cut close to the diaphragm 15 days previously. Cathode-ray oscillographic records. The lines at the left and below represent, respectively, 50μV. and 1 sec. A, left, control phrenic. B, right, test phrenic.

phrenic was cut. For this purpose the records were traced in outline through an enlarger and the average maximal height of the asynchronous discharge estimated for several typical breaths. In the 2 experiments in which no respiratory discharge could be detected from the previously sectioned nerve, the noise level of the record was taken as the maximal discharge. The background noise was 8 to 12μV. in favorable cases, and the respiratory discharges on the control side, while usually 30 to 40μV., occasionally reached 200μV.

The level at which the phrenic was sectioned did not affect significantly the course or severity of the change described. In Fig. 2 the solid rectangles represent animals in which the nerve was cut near the diaphragm, and the open rectangles those in which it was cut at the level of the first rib. The decreased respiratory discharge from the previously sectioned nerve occurred as readily on the right as on the left. When present, it was as marked in the 4th as in the 5th or 6th cervical root of the phrenic nerve. The magnitude of the discharge from a given root did not depend on the intactness of the other ipsilateral or contralateral roots.

Two types of experiment were done to show that it is the interruption of

the axons rather than some other factor in the surgical procedure which leads to the deficiency of respiratory discharge. When the chest wall was opened and the lung was retracted, but the phrenic nerves were not molested, the deficiency did not appear. Moreover, when the chest wall was opened on the right and the left phrenic was cut, or vice versa, the deficiency of respiratory discharge always occurred on the side on which the phrenic had been cut.

In several of the animals in which one phrenic had been cut low in the chest, both phrenics were excised after recording the respiratory discharges and studied in a moist chamber at room temperature. The excitability of the two nerves, one of which had been cut peripherally 1 to 3 weeks previously,

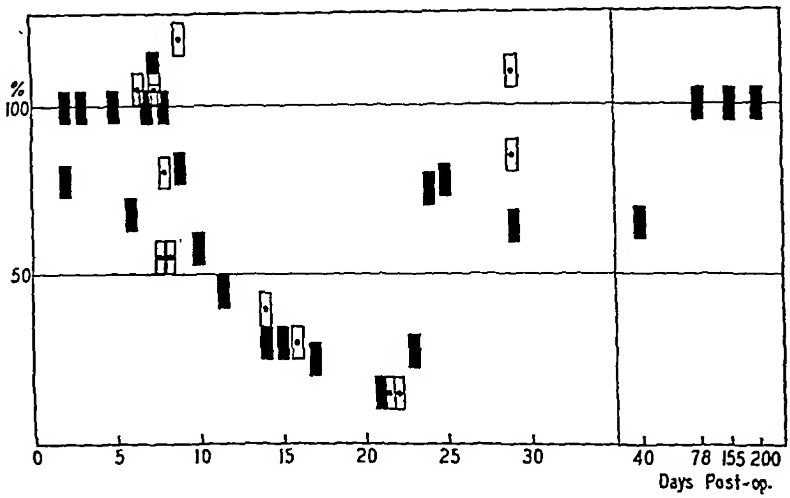

FIG. 2. Chart showing time course of deficiency of respiratory discharge from phrenic nerve following peripheral section. Each rectangle represents a single experiment. Abscissae: days after section of the nerve. Ordinates: amplitude of respiratory discharge from the previously sectioned, test phrenic expressed as per cent of that from the opposite, acutely sectioned, control phrenic. Solid rectangles: test phrenic cut near diaphragm. Open rectangles: test phrenic cut at level of first rib.

was not grossly different. Conduction velocity was usually slower in the previously sectioned nerve. When correction was made for conduction velocity, there was no significant difference in the amplitude of the maximal spike for the A fibers of the two nerves.

Microscopic examinations of the spinal cord in cervical segments 4, 5 and 6 were made in several instances in which the deficiency of respiratory discharge described above was more or less marked (20 to 70 per cent of control). Serial Nissl preparations showed little or no retrograde degeneration in cords from 8 animals in which the phrenic had been cut close to the diaphragm. Bodian and Cajal preparations of these cords also showed little change either in the boutons terminaux or in the neurofibrillae. In one animal

in which the phrenic was cut at the level of the first rib, Nissl preparations showed little or no retrograde changes on the side of the cut nerve.

Discussion

The time course of the deficiency in respiratory discharge described above is strikingly similar to the course of retrograde degeneration in motor neurons, as described by Nicholson (5) and Bucy (2). These authors found relatively mild anatomical changes in the first 8 days following section of motor nerves. From the 9th to the 15th day they describe a marked progressive change in the appearance of the cells, reaching a peak at about 20 days. In the succeeding weeks a gradual return to normal occurs, and by the 40th to 60th day recovery is complete. The physiological change in the phrenic neurons, as tested above by the magnitude of spontaneous discharges, parallels these anatomical changes of retrograde degeneration (Fig. 2). It is interesting to note that Kohnstamm (1899) predicted this relation and suggested that it should be tested on the phrenic nerve.

The fact that the change in function of the neurons follows the same time course as does the anatomical change when the latter is present suggests that a common metabolic process may underlie both. On the other hand, in the present study the phrenic neurons showed little anatomical change (p. 271). Both Kohnstamm (4) and Sano (7), in reporting retrograde degeneration in these neurons, remarked on the mildness of the alterations when compared with what is seen in the nuclei of cranial nerves. Furthermore, the degree of functional loss does not depend on the level of section (p. 271). This result contrasts with the law of retrograde degeneration frequently cited by the older neuro-anatomists, that the farther away from the cell body the axon is cut, the less marked is the anatomical change. These experiments demonstrate that the correlation between the functional and anatomical processes is not strictly quantitative. They further emphasize that for some aspects of the change resulting from section of the axon the functional test is a more sensitive indicator, while, for others, the anatomical method is preferable.

The studies reported above on the excised nerves exclude axonal changes as the cause of the deficiency of respiratory discharge recorded from the previously sectioned phrenic. One can conclude from these data that, short of death of the cell, which sometimes occurs at the peak of retrograde degeneration (6), the conducting mechanism is little affected by the process underlying the other changes. The significance of the decreased conduction velocity remains obscure. The deficiency of respiratory discharge might be due to changes in one or all of the following processes: (i) initiation of nerve impulses in the phrenic neurons; (ii) conduction over the cell body and dendrites of the phrenic neurons; (iii) synaptic phenomena by which the phrenic neurons are excited; and (iv) similar processes in the higher neurons of the ipsilateral respiratory mechanism. The present data do not differentiate among these possibilities.

Summary

After section of the phrenic nerve the respiratory discharges recorded central to the cut undergo a temporary deficiency (Fig. 1) with a time course (Fig. 2) parallel to that described for retrograde degeneration in other neurons. Yet the phrenic cell bodies may show little or no anatomical change even when this deficiency is extreme. The deficiency is not due to failure of conduction in the phrenic axons.

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ORIGIN, CONDUCTION AND TERMINATION OF IMPULSES IN THE DORSAL SPINO-CEREBELLAR TRACT OF CATS

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THE DORSAL SPINO-CEREBELLAR TRACT (Flechsig's tract), which extends from the lumbar cord upward through the restiform body into the cerebellum, presents a favorable object for an electrophysiological study of ascending pathways formed within the cord by secondary neurons. Since it lies just lateral to the line of entry of the dorsal roots and on the surface, the tract is easily accessible to the placement of recording electrodes. It is conspicuous anatomically because a considerable portion of its fibers are large; Häggqvist (7) found that in man about 30 per cent of the myelinated fibers range from 10 to 18μ in diameter. In comparison, only 1 per cent of the fibers of the fasciculus gracilis are larger than 10μ, and of these, furthermore, only a scattered few fall in the range from 11 to 14μ. Flechsig (5), Mott (14), and Van Gehuchten (22), with three different methods established that the larger fibers of Flechsig's tract have cells of origin within Clarke's column (the nucleus dorsalis). Many subsequent workers have confirmed this observation, and also that of Mott (13) that collaterals from the primary sensory fibers of the dorsal funiculus terminate in the vicinity of Clarke's cells. Whether the fibers of Flechsig's tract originate from cells of the ipsilateral, the contralateral, or from both columns has been a subject in question. Discussions of this point are found in Kappers, Huber and Crosby (10), Pass, (15), and Strong, (19). Likewise a matter of differing opinions has been the question of the extent of the distribution of the fibers within the cerebellum (12, 9, 1; and discussions, 10, 11). It is agreed, however, that the fibers of Flechsig's tract end mainly within the cortex of the rostral part of the vermis.

Afferent stimulation of the nerves of the hind limb of the cat, or of the corresponding dorsal roots, initiates electrical activity in Flechsig's tract. This activity has been recorded with electrodes made from steel needles 50 to 250μ diameter in the shank and insulated except for the tip, which is especially sharpened to minimize gross damage to the spinal cord. The most satisfactory electrodes at the present time are 250μ needles ground on a long taper for about 8 mm. and then pointed with a sharp taper to a tip of 5 to 10μ. Needle electrodes so prepared combine mechanical rigidity and ease in insulating with a minimum of destructiveness to the tissue of the spinal cord. The recording and stimulating arrangements used in these experiments are those standard for the laboratory.

The Potentials in Flechsig's Tract

Distribution of potential. The potential recorded from the tract is sharply localized in the dorsal part of the lateral funiculus of the cord, and it is recorded either when the active needle electrode is on the surface of the region or in its most superficial stratum. Often a number of trials have been necessary in placing the needle electrode before the maximum potential could be observed. This is probably correlated with the dispersed nature of the tract as observed in the successive degeneration experiments of Sherrington and Laslett (18) and by MacNalty and Horsley (12). The impulses can be followed from the midlumbar level of the spinal cord into the white matter of the cerebellum.

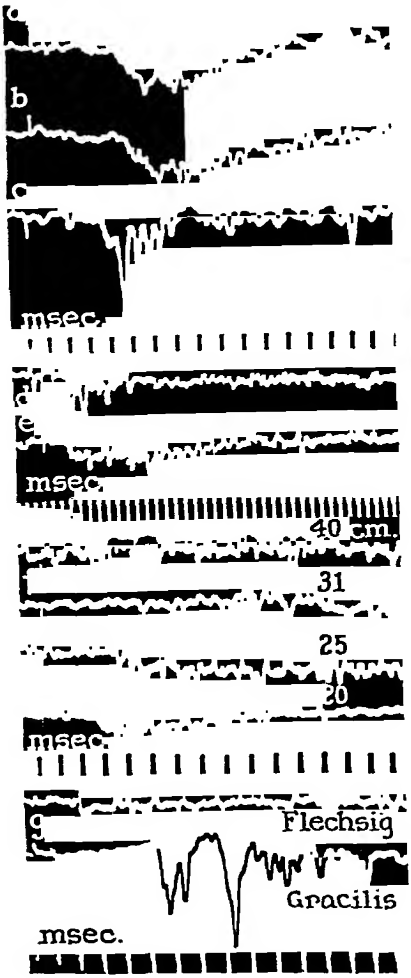
Form of the potential. The electrical activity set up in the tract by a single afferent volley is recorded as a positive potential (Fig. 1, *a* to *e*) which reaches a maximum in 2 to 5 msec. and lasts usually less than 10 msec. In comparison, the potential similarly set up at the same level in the dorsal column is much larger and longer. At the periphery of the dorsal spinocerebellar tract, or in its center if weak stimuli are used, the response often is a series of positive spikes of 0.4 to 0.6 msec. duration (Fig. 7 to 12). These spikes are monophasic and probably represent leads from a few synchronously active fibers.

Axonal properties of spike responses. It cannot be said with certainty which, if any, of the spikes of Fig. 7 to 12 represent activity of individual cells. In some instances the composite nature of the response can be ascertained from the variations in amplitude which it exhibits, but the short duration of these spikes, from 0.4 to 0.6 msec., indicates that the tract axon produces an electrical response which has the same duration as do axonal spikes of peripheral nerve (6).

Effectiveness of various afferent stimuli in producing potentials in the tract. Potentials of similar magnitude and time relations are evoked in the tract by stimulation of the ipsilateral tibial nerve, peroneal nerve, or a dorsal root; and smaller responses are evoked by stimulation of nerves to individual muscles. No impulses are evoked on stimulation of the saphenous nerve, but activity attributable to propriospinal fibers can be recorded in the tract region proximal to the entry zone of the saphenous nerve fibers. Likewise, no impulses are evoked in the tract by stimulation of the contralateral tibial nerve. Flechsig's tract is, therefore, set into activity by fibers afferent for muscle proprioceptors and is uncrossed, but a small crossed component is not ruled out. The evidence for the above experimental findings is shown in Fig. 1.

The distribution of the potentials in Flechsig's tract in response to stimulation of either the tibial or the peroneal nerve appears to be essentially the same (Fig. 1, *a*, *b*). Later, on the basis of the interaction of the responses evoked by the two nerves, it will be shown that there is considerable overlap between the synaptic terminations of the afferent fibers of various nerves on the cells of Clarke's column. The absence of sharp localization in the tract may in part be caused by this overlap.

Conduction of impulses in Flechsig's tract. Figure 2 makes a comparison of the temporal course of the activity in Flechsig's tract with the potential



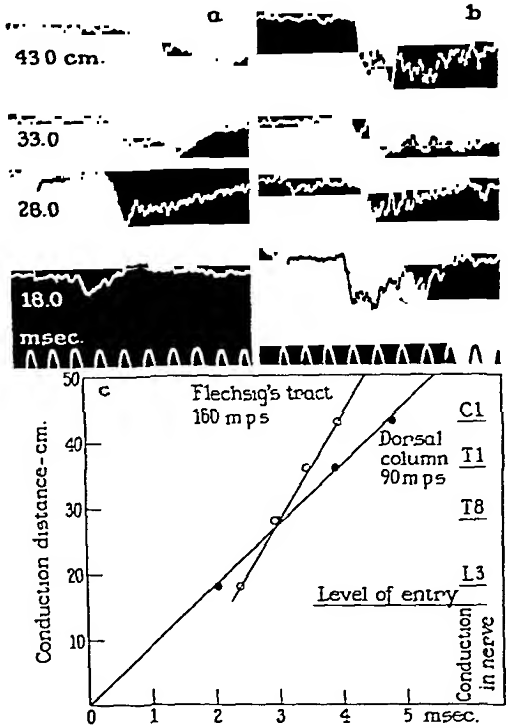


FIG. 2. The temporal course of impulses ascending in the dorsal column and in Flechsig's tract in response to tibial stimulation. *a*, responses in the dorsal column; *b*, responses in the tract at the four conduction distances given on the records. The graphic formulation of the data is given in *c*. ● . . . dorsal column; ○ . . . Flechsig's tract. The ordinates on the left give the distances of conduction; on the right are indicated the approximate levels in the spinal cord.

FIG. 1. Potentials recorded from the dorsal spino-cerebellar tract of the cat, in response to stimulation of the following ipsilateral afferent fibers: *a*, the tibial, and *b*, the peroneal nerve; *c*, dorsal root, SI; *d*, hamstring nerve; *e*, nerve to quadriceps femoris muscle; *f*, saphenous nerve at the four distances of conduction indicated in the figure. In *g*, the upper record is from the tract on stimulation of the contralateral tibial nerve and the lower record from the dorsal column ipsilateral for the nerve, both taken at the same level in the cord.

developed in the dorsal columns as the two sets of impulses ascend in the cord following a stimulus to the ipsilateral tibial nerve. The responses were recorded in both tracts at four distances of conduction (Fig. 2, *a*, *b*). The times at which the earliest response arose in each tract are plotted against the con-

duction distance from the point of stimulation in Fig. 2 *c*. The slopes of the straight lines which join all the points of each set represent velocities with which the impulses are conducted in the two tracts. In the experiment illustrated the conduction velocity is 160 m.p.s. for Flechsig's tract, while in the dorsal column it is nearly half, 90 m.p.s. That impulses are conducted more rapidly in fibers of Flechsig's tract than in the primary afferent neurons

Table 1. Summary of 11 experiments on velocity of conduction of impulses in Flechsig's tract and in dorsal column.

Date	Stimuli applied to:	Rectal temperature (°C.)	No. of points in measurement	Largest conduction distance (cm.)	Conduction velocity (m.p.s.)		Ratio CV Flechsig's tract / CV dorsal column
					Flechsig's tract	Dorsal column	
Dec. 12, '40	Tibial nerve	37.0	4	28	110	56	1.98
Jan. 6, '41	" "	39.5	4	43	160	90	1.78
" 7, '41	" "	37.5	2	43	120	78	1.54
" 21, '41	" "	38.7	5	44	130	75	1.73
" 23, '41	" "	40.0	5	45	125	60	2.10
" 27, '41	" "	37.3	2	24	110	59	1.86
" 28, '41	" "	39.1	6	43	85	50	1.70
" 28, '41	Tracts	39.1	3	21	95	50	1.90
Feb. 5, '41	"	38.0	2	17	103.5	77.5	1.33
" 7, '41	"	39.8	2	16	155	72.5	2.10
" 27, '41	Tibial nerve	37.0	2	38.5	125	64.0	1.95

of the dorsal column was observed in eight experiments, the results of which are summarized in Table I.

The experiments on which are based the data of Fig. 2 and of Table I required some precautions in technique which deserve to be mentioned. The animals were maintained at temperatures within the range of physiological normality. These are enumerated for each experiment in Table I. To avoid cooling the portions of the spinal cord over which the conduction velocity was being measured, the first exposures were always made at the highest levels from which recordings were being taken. The subsequent exposures at lower levels were made in order down the spinal column.

A second method for determining the velocity of conduction was employed in three experiments (Fig. 3, Table I). The dorsal column or Flechsig's tract was stimulated directly at higher levels of the cord and records were made in the lower thoracic and the lumbar cord. Controls showed that the stimulus, when applied to the one tract by means of paired needle electrodes insulated except at the tip, did not spread to the other tract. The velocities of conduction in Flechsig's tract and in the dorsal column obtained in these experiments were entirely concordant with the measurements of the series first cited.

Returning to the records of Fig. 2, it is seen that in the lumbar cord activ-

ity is initiated in the dorsal column before the beginning of activity in Flechsig's tract. At the level of the seventh to the ninth thoracic segments the more rapidly progressing impulses in Flechsig's tract catch up with the activity ascending in the column and then forge ahead to arrive at the upper cervical levels of the cord about one millisecond before the latter. In the course of synaptic transmission in the funicular nuclei, secondary neurons activated by impulses afferent in the columns are further delayed (20). The earliest activity in the medulla is thus a product of the impulses brought to it in Flechsig's tract and not by those carried in the dorsal columns. The

FIG. 3. Comparison between the velocities of conduction of impulses descending in Flechsig's tract (*a*, *c*) and in the fasciculus gracilis (*b*, *d*) in response to direct stimulation of the two tracts at T3. The recording electrode was in L2 for the upper records (C.D. = 13 cm.) and at L4 (C.D. = 16 cm.) in the lower set. The velocities determined from these records are 155 m.p.s., for the fibers of Flechsig's tract and 72.5 mp.s. for those of the dorsal column.

impulses which reach the medulla and the cerebellum via the fibers of Flechsig's tract will be discussed in a later section of this paper.

Relation between diameter of intraspinal fibers and their velocities of conduction. The greater velocities of conduction of the fibers of Flechsig's tract, in comparison with the rates at which impulses are conducted in the dorsal columns, appear to be correlated with the larger size of the fibers of Flechsig's tract. Häggqvist (7) found that 1 per cent of the fibers of Flechsig's tract are 16 to 18μ in diameter. In the dorsal column, on the other hand, while there are some fibers of 12 to 14μ diameter, the group having diameters of 10 to 11μ is the first to amount to 1 per cent of the total number. The ratio of the diameters of the means of the two groups is 17:10.5, or 1.6. The average ratio of the velocities with which impulses are conducted in the fibers of the two tracts is 1.8 (Table I). It appears likely, then, that the velocity with which impulses are conducted within the spinal cord bears the same relation to the diameters of the fibers as that which has been found in the peripheral nervous system (8, 6).

Lower extent of tract. Unambiguous evidence of the presence of Flechsig's tract in the lower lumbar cord is found in the experiments in which the tract was stimulated directly. Figure 3 shows the record of activity in the dorsolateral column of lumbar segment 4 in response to stimulation of Flechsig's tract in the upper thoracic region of the spinal cord. The origin of the tract must, therefore, lie at or below the fourth lumbar level.

In other experiments, responses were observed only at or above the third or the second lumbar segments. This variability is to be expected in view of the known anatomical variability of the level at which Clarke's column is

formed. Beck (1), in degeneration experiments, identified the tract in the fifth lumbar segment.

Origin of Impulses in Flechsig's Tract

Synaptic delay in Clarke's column. Since Flechsig's tract is composed of second order neurons, which have their cells of origin in Clarke's column, a delay is to be expected between the arrival of activity at the collaterals of the afferent primary neurons and the onset of post-synaptic activity in Clarke's cells. This delay appears most probably to account for the interval between the appearance of the response in the dorsal column and in Flechsig's tract which is shown in the records of Fig. 2 *a* and *b*. The synaptic delay can be measured from the graph in Fig. 2 *c*, on the assumption that in the animal of this experiment the origin of the tract occurred at the fourth lumbar segment, as is indicated in the figure. The delay so measured is 0.55 msec.

Another method has also been employed to measure the magnitude of the synaptic delay (Fig. 4). In the lumbar and lowest thoracic levels of the cord, the large potential which represents the activity of Flechsig's tract is preceded by a smaller potential which begins synchronously with the onset of activity in the dorsal column at the same level. It is interpreted by us as the direct pick-up of the potential from collaterals of the fibers of the dorsal column to the region of Clarke's column. The interval between the start of this small potential and the tract response is then the interval of the synaptic delay at Clarke's column. The synaptic delay measured from the records of Fig. 4 ranges from 0.5 to 0.9 msec.

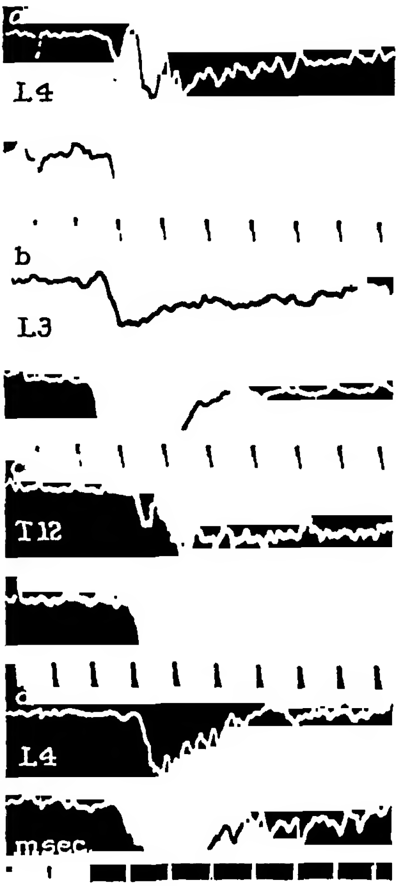


Fig. 4. Activity recorded in Flechsig's tract (upper record of each set) and in the dorsal column (the corresponding lower record) on stimulation of the ipsilateral tibial nerve. The recording electrode was in the lowest thoracic or in the lumbar levels of the spinal cord, as indicated on the records. The response occurred earlier in the dorsal column than in Flechsig's tract by 0.5 to 0.9 msec. Ahead of the tract response is seen a small deflection in the tempo of the onset of activity in the column.

Synaptology of Clarke's Column

The degree of complexity in the synpatic connections of Clarke's cells is at present unknown in terms of anatomical data. Some indication of their activity is, however, obtainable on the basis of the electrical records. In

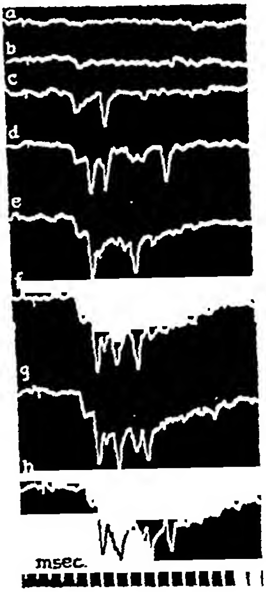


FIG. 5. Response in Flechsig's tract to progressively stronger stimulation of the tibial nerve. At the strength of stimulation used in *a*, no response was elicited. With somewhat stronger stimuli, the first response was small, *b*. This increased with stronger stimuli, *c*, but thereafter remained essentially unchanged in later records with still stronger stimulation of the nerve. Another group of elements, recorded as a sharp spike, made its appearance in *c*. With stronger stimuli to the nerve, the first spike response arose earlier and was followed by more spikes and other, less well-defined activity, *d* to *h*.

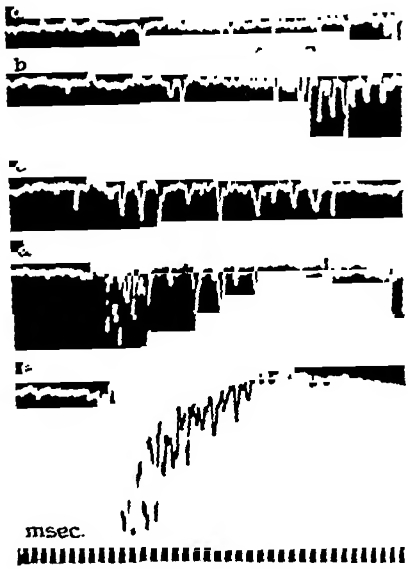


FIG. 6. Another experiment showing the modification of the responses in the tract by progressively increasing strength of stimulation of the tibial nerve. *a*, *b*, very weak stimuli, *b* the stronger. The late activity in *b* may be spontaneous in origin, but is in the pattern of the response to stimulation in the subsequent records. *c*, *d*, further increases in the strength of the stimulus. With the stronger, *d*, the multiple response arose earlier, was larger, and the spikes were more closely grouped. Response to supramaximal stimulation of the nerve is shown in *e*.

almost every experiment it has been found that the micro-electrode can be so placed in the tract as to record a series of discrete spikes. When such spikes are recorded, their number can often be changed by varying the

strength of the afferent stimulus. A weak stimulus to the nerve produces one or a few spikes, and these increase in number and in frequency progressively as the stimulus is strengthened (Fig. 5). The interval between the stimulus and the first appearance of the spikes may also be decreased as the stimulus is increased (Fig. 6). *These several observations indicate that there is no one-to-one relationship between fibers of the dorsal column and Clarke's cells, that sensory fibers of different electrical thresholds activate the cells, and that there is probably a multiple order of synpatic activation of the cells.*

Interaction on the responses in Flechsig's tract between the effects of stimulating different nerves gives more specific information on these findings. This is particularly the case in experiments in which the activity elicited under the recording micro-electrode takes the form of discrete, identifiable spike potentials.

Convergence of excitation from afferent fibers. In the experiments of Fig. 7, a stimulus to the peroneal nerve caused in the tract a typical response in the form of three spikes, each lasting 0.5 to 0.6 msec. (records *a*, *b*, *c*). The form and amplitude of the three remained remarkably constant throughout the experiment and persisted through several small displacements of the micro-electrode. This last in itself indicates that the potentials were being conducted in a group of homogeneous fibers which had been nearly uniformly activated by a pool of cells in Clarke's column. The time of arrival of the first spike remained constant throughout the experiment at 2.4 msec. after the shock. The second, smaller spike followed the first by a constant interval of 0.9 msec. The third spike, in amplitude equal to or greater than the first spike, followed the first by an interval of 4.2 msec. in the majority of the records of the figure. This interval varied considerably, becoming shortened to 4.0 msec. (records *a* and *q*), and in two records (*c* and *t*), it was diminished by 0.6 msec.

Only a single spike was elicited on stimulating the hamstring nerve. This arrived at the electrode after the same interval as did the first response to stimulation of the peroneal nerve. In form the two spikes were also closely similar, but the response to the hamstring nerve could be equal to, or greater or smaller than the first response to the peroneal nerve (records *d*, *k*, *r*). This observation indicates that the spike in response to stimulation of the hamstring nerve was also the product of the simultaneous activation of a pool of Clarke's cells, and that the impulses were conducted in the tract in a homogeneous group of fibers.

The two early spikes, caused by the stimulation of either nerve, were due to the activation of the same pool of Clarke's cells by the collaterals of fibers afferent in either of the two nerves. This is shown by the results of applying both stimuli at intervals varying from 1.2 to 1.8 msec. (records *e* to *i*). The single spike caused by stimulation of the hamstring nerve was completely inhibited when there was activity at the recording electrode due to the first "peroneal" spike and for well over a millisecond later. The response was partially inhibited at later intervals (records *l*, *m*). The available data do

not permit identification of the third spike of the peroneal response with the activity of the same pool of cells. Inspection of records *p* to *u* indicates, however, that this is probable.

Number of steps involved in the response. The time relations of the triple spike response to peroneal stimulation (Fig. 7) yield information on the

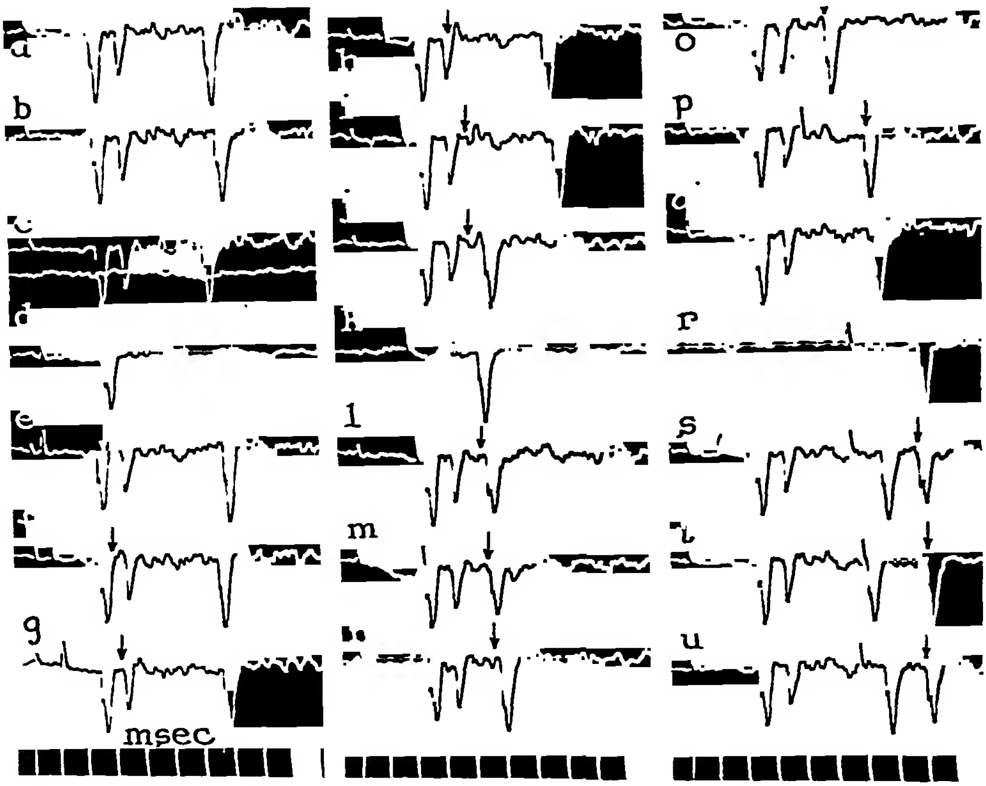


FIG. 7. Interaction between the responses evoked in the dorsal spino-cerebellar tract by stimulation of the peroneal and the hamstring nerves. The response to stimulating the peroneal nerve alone is seen in *a, b, c, q;* that evoked by stimulation of the hamstring nerve alone is shown in records *d, k, r.* Arrows indicate the expected time of onset of the test response. (Further description of the records is in the text.)

activity which may be involved in the response of Clarke's cells. The first spike in response to peroneal stimulation and the single spike caused by a stimulus to the hamstring nerve represent activation of the Clarke's cells by the direct collaterals of the primary sensory fibers afferent in the two nerves. The responses occurred 2.4 msec. after the stimuli, or 0.6 msec.—a synpatic delay—after the afferent volley had reached the dorsal column at the same level of the spinal cord. The second of the spikes in the response to peroneal stimulation, which occurred 0.9 msec. after the beginning of the first spike, may have been the product of activation of Clarke's cells through

an intercalated neuron, through direct impulses in collaterals of slowly conducting afferent fibers, or through the combined action of impulses reaching the cells from both sources. The third spike of the response most probably was the product of internuncial activity. It arose late, when most of the

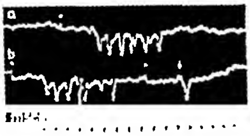


FIG. 8. Inhibition of responses which are mediated by internuncial activity. A single stimulus to the tibial nerve produced a series of spike responses in the tract, *a*. All but the first of these was inhibited by the conditioning effect of activity initiated through another stimulus to the tibial nerve 11 msec. earlier, *b*. The stimulus artifacts are recorded by dots.

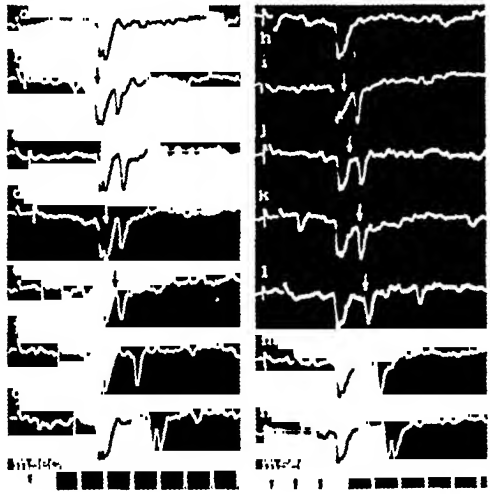


FIG. 10. Responses of Clarke's cells to internuncial activity set up by summation of stimuli to different nerves. *a*, the response to stimulation of the tibial nerve, and *h* to stimulation of the peroneal nerve. In records *b* to *g* and *i* to *n*, both stimuli were applied together, the tibial stimulus leading in the records of the left column, and the peroneal stimulus leading in the column on the right. The extra responses of records *b* to *d* and *i* to *k* were set up by internuncial activity initiated by the summation of excitation via the two afferent volleys. The time at which the onset of the response to the second stimulus was to be expected is marked with an arrow. (Further description of the records is in the text.)

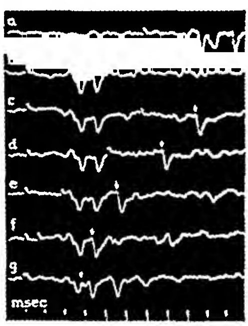


FIG. 9. Differential effects of antecedent conditioning activity on the primary and internuncial activation of Clarke's cells. Same preparation as for the preceding figure, but with the conditioning and testing responses produced by stimuli applied to different nerves. *a*, response in the tract to stimulation of the tibial nerve. *b*, the response, at the same locus, to stimulation of the peroneal nerve. *c*, *d*, *e*, the "tibial" response preceded by conditioning "peroneal" activity. Only the first spike of the test response survived. *f*, this spike fell into the refractory period produced by the second spike of the peroneal response. *g*, the two stimuli were applied within 0.2 msec. of each other. An extra response occurred in the tempo of the third spike of the tibial response of record *a*. The arrows indicate the expected time of onset of the first spike of the test response.

activity in the primary volley had subsided. The stimulus-response time varied by a small amount (0.2 msec.) in the majority of the records, a variation which may be ascribed to the known variability of the synpatic delay. A larger variation (0.6 msec.; records *c* and *t*) probably represents a shortening of the internuncial chain by one link.

Inhibition of responses which involve interneurons. When the "hamstring" spike was able to develop ahead of the third "peroneal" spike (Fig. 7; records *j* to *p*), the latter was inhibited. The inhibition may have been produced by one of two causes. If the third "peroneal" spike were the result of activation of the same group of Clarke's cells, the "hamstring" spike had left these cells in a state of refractoriness too great to be overcome by the activity of the interneurons which mediated the later spike. It is also possible that the collaterals of the fibers afferent in the hamstring nerve, while unable to initiate a pattern of internuncial activity which would lead to the stimulation of the Clarke's cells, nevertheless were able to break up the pattern initiated by the collaterals of the peroneal afferents, and thus to inhibit the late "peroneal" spike. Whatever the cause, activity in Flechsig's tract which is mediated by interneurons is readily inhibited.

Activity which is set up in Clarke's cells through internuncial chains may be inhibited for a considerable time. Eleven msec. after a stimulus to the tibial nerve had set up a volley of spike activity in the tract (Fig. 8 *b*) a second stimulus to the tibial nerve elicited only the first of the series of spikes, that caused by the activation of the Clarke's cells by direct collaterals from the primary neurons.

Inhibition of activity mediated by interneurons occurs also when the internuncial activity is initiated through volleys in different afferent paths. In the experiment of Fig. 9, stimulation of the tibial nerve caused a triple spike response in the tract (*a*). Stimulation of the peroneal nerve caused only two spikes (*b*). On conditioning the tibial response with antecedent peroneal activity, the last two spikes of the tibial response were inhibited completely (*c* to *e*). There remained only the first spike, that due to the stimulation of the Clarke's cells by the collaterals of the primary afferent fibers. This spike also was absent when its onset fell into the refractory period set up by the antecedent responses to peroneal stimulation (*f*, *g*), showing that the same elements were active in response to stimulation of either nerve.

Excitation by summation of stimuli. Figure 10 shows an experiment in which stimulation of two nerves within the summation interval evoked an extra response in the tract.

The response to stimulation of the tibial nerve (record *a*) or of the peroneal nerve (record *h*) was in this experiment a compound of several spike responses. It was longer than the minimal duration observed in other experiments, and appeared with a double peak of positivity, sometimes nearly fused and sometimes more distinct. Two components were clearly seen when the response was evoked by stimulation of one nerve against a background of conditioning activity set up via the other nerve (records *g*, *n*). The stimulus-response interval differed for the two nerves, being 3.4 msec. for tibial (*a*) and 2.95 msec.

for peroneal (*h*) stimulation. This difference may have been due to difference in the conduction time in the primary neurons and in the synaptic delay. It served to determine the origin of the response when the two stimuli were pitted one against the other.

Stimulation of both nerves so as to cause the evocation of both responses simultaneously (the peroneal stimulus lagging by 0.45 msec.) caused a combined response (*b*), the early part of which was no larger than that produced on stimulation of the tibial nerve alone. The activity set up by stimulation of either nerve was, therefore, in a common group of Clarke's cells. A delayed, additional response was also set up 0.75 to 0.85 msec. after the beginning of the first response. The time of onset of the extra response remained constant within this range when the two stimuli were separated by a considerable interval, whether the tibial stimulus was leading (*c* to *e*) or the peroneal (*i* to *l*). The extra spike was the response of Clarke's cells to stimulation of interneurons which were brought into play by the combined effects of two primary volleys when these were separated by no more than the summation interval. The summation interval measured from this experiment is 0.5 msec. When the interval between the stimuli was greater than the summation interval, the responses were to synaptic stimulation by the direct collaterals of the primary neurons. At first, only the early group of Clarke's cells recovered sufficiently to respond a second time (*f*, *m*); then the late group also recovered (*g*, *n*).

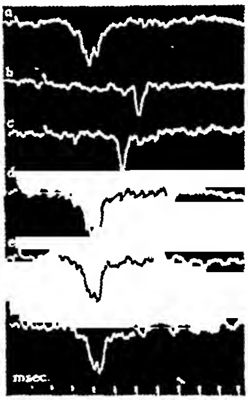


FIG. 11. Responses set up by internuncial activity. *a*, response in Flechsig's tract to stimulation of the tibial nerve. The stimulus-response time was 2.6 msec., indicating direct stimulation of Clarke's cells by collaterals of the primary afferent neurons. *b* and *c*, successive responses to stimulation of the peroneal nerve. These occurred 4.25 and 3.3 msec. after the stimulus and involved internuncial activity of variable degrees of complexity. The subsequent records show complete inhibition of the response evoked through internuncial stimulation by conditioning via the tibial nerve.

In this experiment it was not possible to ascertain whether the response set up by the interneurons activated within the summation interval represented the activity of the group of Clarke's cells stimulated by the primary neurons, or whether it was due to stimulation of another, previously inactive group. The experiment of Fig. 11, however, shows clearly that cells which are not activated by direct collaterals can be set into activity through internuncial chains of varying length.

In this experiment, stimulation of the peroneal nerve caused only a spike mediated by internuncial activity. This can be ascertained by comparing the stimulus-response time for the tibial response (*a*, 2.6 msec.) with the time for the spikes evoked via the peroneal nerve

(4.25 msec., *b*; and 3.3 msec., *c*). The response of record *b* probably involved two interneurons, while in *c* the chain was shortened to the participation of only one intereuron. As in other experiments described earlier, these responses, which involve internuncial activity, were completely inhibited by antecedent stimulation of the tibial nerve (*d* to *f*).

The foregoing series of experiments indicates that Clarke's cells can be activated by collaterals from a number of primary neurons and by internuncial chains of varying length, through which the impulses from a number of primary pathways converge. Cells not stimulated by one set of primary impulses and the interneurons activated by these may be brought into activity by summation with another set; and conversely, the responses of cells that would be stimulated by one set may be inhibited by another set.

Independence in the activity of other cells of Clarke's column is shown in the records of Fig. 12. Stimulation of the tibial nerve (*a*) and of the per-

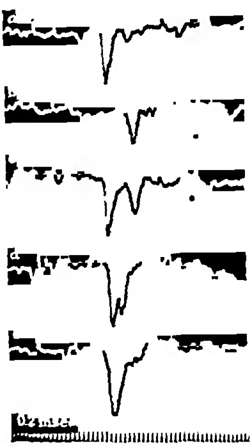


FIG. 13. The effect of previous activity on the large tract response. *a*, the response in the tract to a single stimulus to the tibial nerve. *b*, the second of two responses is diminished in size and duration.

FIG. 12. Independent activity in Clarke's cells in response to stimulation of two nerves. *a*, tibial, *b*, peroneal nerves stimulated individually. *c*, *d*, *e*, both nerves stimulated in rapid succession.

oneal nerve (*b*) produced responses of different forms at the recording electrodes. Combination of the two responses resulted in their addition (*c*, *d*, and *e*).

The maximal response. The information obtained under the special conditions where discrete or identifiable groups of responses were recorded serves also for an understanding of the behavior of the large composite response usually recorded in the tract. Since repetitive activity can occur at rates above 500 per sec. (Fig. 7, 10) the response to a maximal afferent stimulus is little affected in its early portion when it is preceded by previous conditioning activity initiated through the same nerve (Fig. 13). Later activity, on the other hand, is considerably reduced. At least in part this may be caused by the inhibition of responses mediated by interneurons. It is also probable that some of the later responses to a single afferent volley are produced by the restimulation of Clarke's cells when the primary afferent neurons are reactivated in the phenomenon which produces the dorsal root reflex discharge (21). Since the reactivation of the dorsal root fibers is easily inhibited by previous activity, this repetition of primary activity will be absent in the

second afferent bombardment of Clarke's cells and will result in the absence of responses in the tract.

The foregoing formulation of the synaptology of Clarke's column is based on electrophysiological data. It calls for internuncial chains of some complexity. No anatomical evidence either for or against the existence of such chains to Clarke's cells is available. The profuse fibrillar network which surrounds the cells is usually considered as formed by collaterals from the primary afferent fibers (23, 2). However, Schäfer (16) described degenerating fibers among the cells of Clarke's column subsequent to lesions of pyramidal fibers. Unfortunately, only the brief statement of this observation was published at a date when the importance of the propriospinal neurons was not yet appreciated (17).

Termination of Flechsig's Tract

Potentials in upper levels of tract. On their course to their final destination in the cerebellar cortex the impulses which ascend in the tract can be observed at the level of the obex (Fig. 14 *b*), in the restiform body (*c*), and in the white matter of the cerebellum (*d*). Activity in the cerebellum starts more than one millisecond before the time at which impulses in the dorsal column (*a*) arrive at the level of the obex.

FIG. 14. Potentials in response to stimulation of the ipsilateral tibial nerve recorded from: *a*, fasciculus gracilis, and *b*, Flechsig's tract, at Cl; *c*, in the restiform body, and *d*, in the white matter of the cerebellum. In *d*, the stimulus artifact is at the beginning of the record.

Distribution of fibers in cerebellar cortex. The distribution of the dorsal spino-cerebellar fibers within the cortex of the cerebellum has been obtained by mapping the electrical responses recorded at various points of the cerebellar surface when Flechsig's tract is set into activity. In the experiments most satisfactory for this purpose, the tract was stimulated directly in the lower thoracic region of the spinal cord. On the direct stimulation of the tract, responses were recorded from the rostral portion of the vermis after a short conduction time, which in the various experiments corresponded to velocities of conduction of 100 to 140 m.p.s. These are in the range of the velocities for the conduction of impulses in Flechsig's tract which have been measured in other types of experiments and described in a foregoing section.

The mapping of the cerebellar surface has been most extensively carried out on the dorsal and rostral faces of the vermis. The early potential which corresponds to the direct activity of tract fibers is confined to the vermis and is largest in the lobulus centralis and in the immediately adjacent folia of the culmen. There is relatively little modification in the amplitude of the potential on shifting from the midline to the ipsilateral border of a folium.

Somewhat smaller, but nevertheless definite, potentials are also recorded from the contralateral halves of the folia, but they do not extend so far laterally as do the ipsilateral potentials. The distribution of dorsal spinocerebellar fibers in the cerebellar cortex of the vermis found in the mapping experiments is shown by the shading in the diagram of Fig. 15.

In these experiments less extensive but equally definitive data were obtained which show that few, if any, fibers of the tract go to the declive, or to the pyramis of the vermis, and that none goes to the cerebellar hemispheres.

The cortical distribution of the dorsal spino-cerebellar fibers indicated by these electro-physiological mapping experiments confirms in the main that given by MacNalty and Horsley, Ingvar, and Beck. Our data indicate that fewer of the fibers are distributed to the culmen than was believed by these authors, and none or only a few to other portions of the vermis, in which degenerating fibers have been seen after sectioning the tract. Beck stated that the majority of the fibers fan out laterally and that few or none crossed the midline. The electrical records show that a considerable number of fibers from Flechsig's tract arrive at the midline and that of these a number sufficient to produce recordable activity cross to the opposite side.

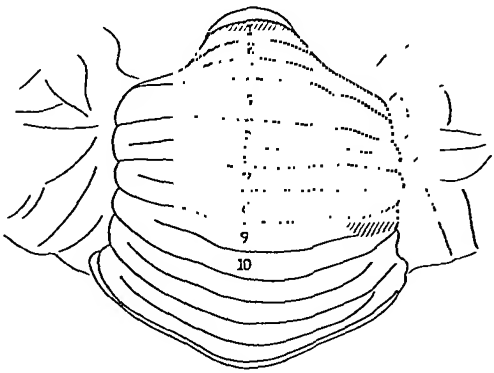


FIG. 15. Diagram showing the bilateral distribution of fibers from the right Flechsig's tract in the lobulus centralis and nearby folia of the culmen of the vermis. The shading represents the region to which fibers are distributed as found from electrical mapping experiments. The numbering of the folia is arbitrary, No. 1 being the first observed above the floor of the fourth ventricle.

Potentials from cerebellar cortex. Considerable exploration has been made of the cortex of the cerebellum in order to observe the electrical response in it resulting from the afferent stimulation of the nerves of the hind limb. The details of these potentials are the subject of continuing investigation and will be omitted here, except in so far as pertains to information on the cerebellar effect of the impulses carried in Flechsig's tract.

In response to afferent stimulation of the tibial or peroneal nerve, the activity recorded from the surface of the ipsilateral rostral portion of the vermis has the simple form shown in Fig. 16. It is essentially identical with the potentials described by Dow (3). The activity is a prolonged positivity which is clearly made up of three components. Other, later components are also demonstrable, but here attention will be centered only on the first three.

The earliest part of the electrical activity occurs 4.0 to 6.0 msec. after the stimulus in the various preparations. Following it there is a second potential at 8 to 10 msec. The dominant potential of the surface electrical re-

sponse is usually the third, which begins at 13 to 17 msec. after the stimulus. This is the potential best seen in the figures of Dow's paper, in which the

FIG. 16. The electrical activity of the cerebellum in response to stimulation of the ipsilateral tibial nerve, recorded with a needle electrode just touching the surface of the cortex. Three components are clearly seen in the electropositive response and other, later components are also indicated. Further description is in the text.

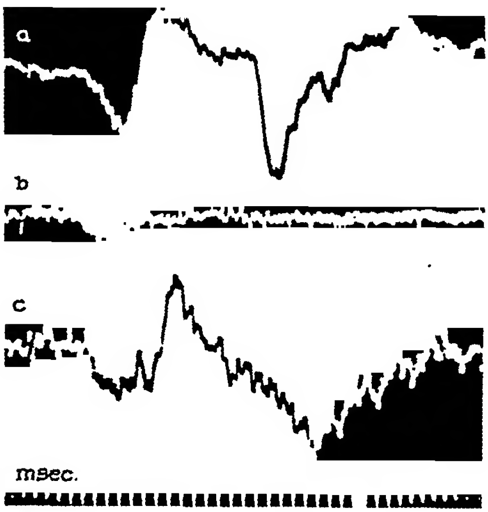


FIG. 17. Modification of the cerebellar response to afferent stimulation, *a*, by asphyxia, *b*, and its restoration following recovery from asphyxia, *c*. Recording needle electrode on the lobulus centralis of the vermis. In the control, *a*, the second potential is negative and the later potentials are positive. During asphyxia, *b*, only the first component of the normal response remains. Upon recovery from early asphyxia, the later components return, *c*. In this record, the second potential begins positive and ends from a negative level.

FIG. 18. Cerebellar potentials recorded from the surface of the lobulus centralis of the vermis to stimulation of the ipsilateral tibial nerve *a*. The first positive deflection begins at 4.6 msec. and is succeeded, at 9.7 msec., by a second potential which in this case is also positive. At 13 msec. another large potential is added to the second. Only this last potential remains after Flechsig's tract has been sectioned at T9, *b*. The cerebellar potential of the opposite side in response to stimulation of the other tibial nerve is shown in *c*. All the components of the normal cerebellar potentials are present as in *a*. The second potential component is smaller than in *a*. Its initial positivity goes over into negativity. The third potential is large and is followed by still later components.

records were made at a slow rate of traverse of the electron beam. When the recording needle electrode penetrates the surface of the cortex, the observed response undergoes numerous modifications. These modifications are to be borne in mind when Fig. 16 to 20 are examined. While the magnitudes and signs of the components of the potentials vary in these records, the basic pattern is nevertheless evident.

The first component of the cerebellar potential, as is indicated from its early time of onset, represents the direct activity of the fibers afferent in Flechsig's tract. Further proof of this has been obtained in two ways. Ischemia of the cerebellum, or the early stage of asphyxia of the animal, abolishes all but the first component of the cerebellar potential. This persists long after the disappearance of the later components (Fig. 17, *b*). On

Fig. 19. Cerebellar activity evoked at one locus on stimulating different afferent nerves. At this locus, the first two components of the activity produced by stimulating the ipsilateral tibial nerve, *a*, are negative. Only late potentials of cerebellar response are appreciable on stimulation of the ipsilateral saphenous nerve, *b*.

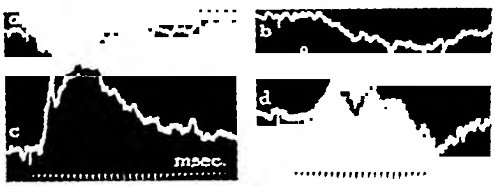


Fig. 20. Cerebellar potentials recorded from the surface and from within the cerebellar cortex on direct stimulation of Flechsig's tract, *a* and *c*, and in response to stimulation of the ipsilateral tibial nerve, *b* and *d*. The upper record of each set is of the potential from the surface.

recovery from early asphyxia, the late potentials return (*c*), but if the asphyxiation is continued farther, the first potential is also abolished at a time when activity still persists in the dorsal columns.

A stricter localization of the early potential of the cerebellar reponse to impulses afferent in Flechsig's tract is made possible by the second type of experiment (Fig. 18). By transecting the ipsilateral dorsal spino-cerebellar tract, the normal cerebellar response to stimulation of the tibial nerve, *a*, is modified, *b*, in that the early potential disappears and that the second component is greatly reduced. Both these components are still present in the response of the opposite cerebellar cortex to its ipsilateral afferent stimulation, *c*.

Since the second component of the cerebellar response is either greatly reduced or abolished when Flechsig's tract is sectioned, this potential must represent the activity of cerebellar units which are in large proportion stimulated by impulses afferent in the tract. On the other hand, the persistence

under the same conditions of the late component indicates that the cerebellar elements which cause it are activated independently of impulses from the dorsal spino-cerebellar tract.

On this point additional evidence comes from a comparison of the cerebellar responses obtained on stimulating the ipsilateral tibial and saphenous nerves (Fig. 19). The earlier potentials of the response to tibial stimulation (*a*) are absent in the activity set up by stimulation of the saphenous nerve (*b*).

Cerebellar potentials in response to direct stimulation of Flechsig's tract. A large, relatively undispersed volley of activity arrives at the cerebellar cortex when the tract is stimulated directly. Stimulation of a peripheral nerve, on the other hand, produces a more dispersed volley in the tract. This is prolonged by the repetitive activation of Clarke's cells and accompanied, during the late activity, by impulses which arrive via other pathways. Therefore, differences which appear in the cerebellar responses set up by the two types of afferent volleys throw further light on the cerebellar effects of activity in Flechsig's tract.

As recorded from the surface of the cerebellum, the electrical response is positive (Fig. 20*a*), but short in comparison with the potentials evolved on stimulating the tibial nerve, *b*. The difference appears to be due mainly to the absence in *a* of the late components of the cerebellar response observed when the tibial nerve is stimulated. The positivity recorded in *a* becomes converted into a large negative potential when the tip of the needle electrode penetrates the surface of the cortex, *c*. At the same locus the response to tibial stimulation, *d*, shows an early negativity and a large later positive potential. This positivity has the time relation of the third component of the surface potential in *b*. Its absence from the response to direct stimulation of the tract furnishes more proof that the cerebellar units which produce this potential are activated independently of impulses from Flechsig's tract.

These observations are in agreement with the experiments of Ferraro and Barrera (4), who found considerable neurological disturbances in the macaque monkey following sectioning of the dorsal spino-cerebellar tract. The disturbances became more severe when other tracts afferent to the cerebellum were also destroyed at the same time.

Summary

By following the electrical responses set up in the dorsal spino-cerebellar tract of the cat, information has been obtained on the anatomy and physiology of the tract, on the synaptology of their cells of origin, presumably lying in Clarke's column, and on the activity which is initiated within the cerebellar cortex by impulses afferent to it in Flechsig's tract. Activity in the tract has been caused either by stimulation of the afferent fibers of peripheral nerves of the hind limb or by direct stimulation of the tract.

Flechsig's tract extends from the fourth lumbar level of the spinal cord into the vermis of the cerebellum. It is set into activity by impulses in the

primary neurons of ipsilateral nerves which are afferent for muscle proprioceptors.

Impulses are conducted in Flechsig's tract nearly twice as rapidly as in the fasciculus gracilis. The greater conduction velocities in the tract are correlated with the presence of larger fibers.

Activity arises in Flechsig's tract after a delay of 0.5 to 0.9 msec. following the arrival of the primary impulses in the collaterals to Clarke's column. As a consequence of the greater conduction velocity in the tract, however, the delayed impulses overtake and pass the impulses which ascend in the fasciculus gracilis. They arrive at the medulla more than one millisecond earlier than the latter.

The responses of fibers of the tract are spikes lasting about 0.5 msec. To a single afferent volley, the response is often a series of such spikes. The fibers are able to respond again within 2.0 msec. after the beginning of an earlier response. The response evoked on stimulation of one afferent pathway frequently is conditioned by activity resulting from stimulation of another afferent nerve. The conditioning effect may take the form of inhibition, or the afferent impulses may sum their excitatory effects. The electrical data indicate that the cells of origin of the fibers of the tract receive collaterals from more than one primary sensory neuron, and evidence also points to their activation by internuncial chains of varying degrees of complexity.

In the cerebellar cortex, the fibers of Flechsig's tract are distributed to the lobulus centralis and to the adjacent folia of the culmen, principally on the same side, but with an overlap across the midline to the opposite side.

Impulses which reach the cerebellar cortex via fibers of Flechsig's tract initiate considerable electrical activity in the cerebellum. Early components of the cerebellar response to all the afferent systems activated by a volley to the tibial or peroneal nerve have been identified as largely, or entirely due to the mediation of impulses from Flechsig's tract. Another component has been shown to be produced independently of activity introduced via Flechsig's tract.

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INTERFERENCE FACTORS IN DELAYED RESPONSE IN MONKEYS AFTER REMOVAL OF FRONTAL LOBES*

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DURING THE PAST DECADE a number of investigations by Jacobsen and his coworkers have employed various tests to study the effect on behavior of complete bilateral destruction of the frontal association areas in primates. These methods have included delayed response tests, delayed alternation tests, stick problems, peg problems, puzzle boxes, and tests of discrimination of visual pattern, brightness, size, and temporal intervals.

The delayed response problem has shown the clearest change in performance after operation. The procedure involved three successive parts: (i) cue presentation, (ii) delay interval, and (iii) the time of choice. The animal watched the experimenter put food under one of two inverted cups. At the end of the delay interval the animal was permitted to raise one of the cups and receive food if it chose the baited cup. With normal monkeys delays longer than 90 seconds were obtained; operated monkeys were unable to perform successfully with delays as short as 5 seconds. Jacobsen states (11, p. 43) that this loss of capacity for delayed response is total and permanent.

On the other hand, the operated animals were successful in all the tests of discrimination learning. In discrimination learning the animal made a choice between two stimuli which remained present throughout any one trial. The animal pulled a drawer underneath one of the stimulus objects, and choice of the positive stimulus object was rewarded with food. Jacobsen (10) found that two of three operated monkeys showed good retention of discrimination habits learned preoperatively (while the other animal showed impairment, but not complete loss). He states that there was no reduction in the ability of the animals to acquire new discrimination habits. Finan's experiments (4) have shown that temporal discrimination is still possible after operation.

Extensive ablations in other cortical regions caused no deterioration of the ability for delayed response. This was shown for the *temporal* lobes by Jacobsen and Elder (12), for the *parietal* lobes by Jacobsen (11), for the *postcentral convolutions* by Breslaw, Barrera, and Warden (1) and for *motor* and *premotor* areas by Jacobsen and Haslerud (13). To this extent it has been

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shown that the defect in delayed response performance following removal of the frontal association areas is specific to damage of those areas alone.

Jacobsen suggested the hypothesis that the frontal lobes were essential for immediate memory. He suggested, moreover, that there may be a real dichotomy between phenomena of memory span and of learning by repeated trials—a dichotomy in the sense that they are two discrete processes, mediated through different neurological mechanisms.

Nissen and his coworkers have emphasized the learning aspect of delayed response and have suggested (18, p. 382) that delayed response is impossible for operated animals because they fail to form associations during the pre-delay period. They pointed out that the delayed response situation is a series of *learning-retention trials: learning takes place at the time food is shown in* one cup; retention is tested at the end of the delay interval. The operations which define delayed response (or one-trial learning) are that the learning must occur in one trial without differentially administered rewards or punishments. Their hypothesis is that only animals with intact frontal lobes are capable of one-trial learning.

This analysis is, however, just as applicable to the retention as to the learning aspect of delayed response. Their definition implies two differences between delayed response and discrimination learning: (i) single vs. repeated trials, and (ii) secondary vs. primary reward. In the pre-delay learning, the *sight* of food is a *secondary* reward because it is a stimulus which derives its reinforcing value from prior conditioning in which it has been associated with a primary reward (7, p. 350). Delayed response, on both counts, is the more fragile habit. It would be in line with the interference theory of forgetting to suppose that performance on delayed response would be more deleteriously affected by activities occurring between learning and recall than would performance on discrimination learning which is a less fragile habit.

In Jacobsen's experiments the degree of *extraneous* visual stimulation was uncontrolled. Response to such extraneous stimuli may have led, therefore, to activity which interfered with the retention of the more fragile habit (delayed response). The present experiment is designed to control the degree of visual stimulation occurring during the delay interval of delayed response in order to determine (i) whether elimination of visual stimulation during the delay period will lead to high scores on delayed response by operated animals and, if so, (ii) whether interpolated visual stimulation will affect the performance adversely. In the present experiment the indirect method of delayed response was used: light stimuli instead of food stimuli served as cues.

APPARATUS

Subjects. The animal subjects used in this investigation were one female rhesus monkey (*Macaca mulatta*) and one male mangabey monkey (*Cercocebus torquatus*). Details of age, adaption to experimental procedures, etc., are presented in the protocols for individual subjects.

Apparatus. A dark room (dimensions: 2.9 $\times$3.3 meters) was used for these experiments; a dim light on a desk served for record-taking. The only lights visible to the animals were the stimulus lights; between trials they were in darkness.

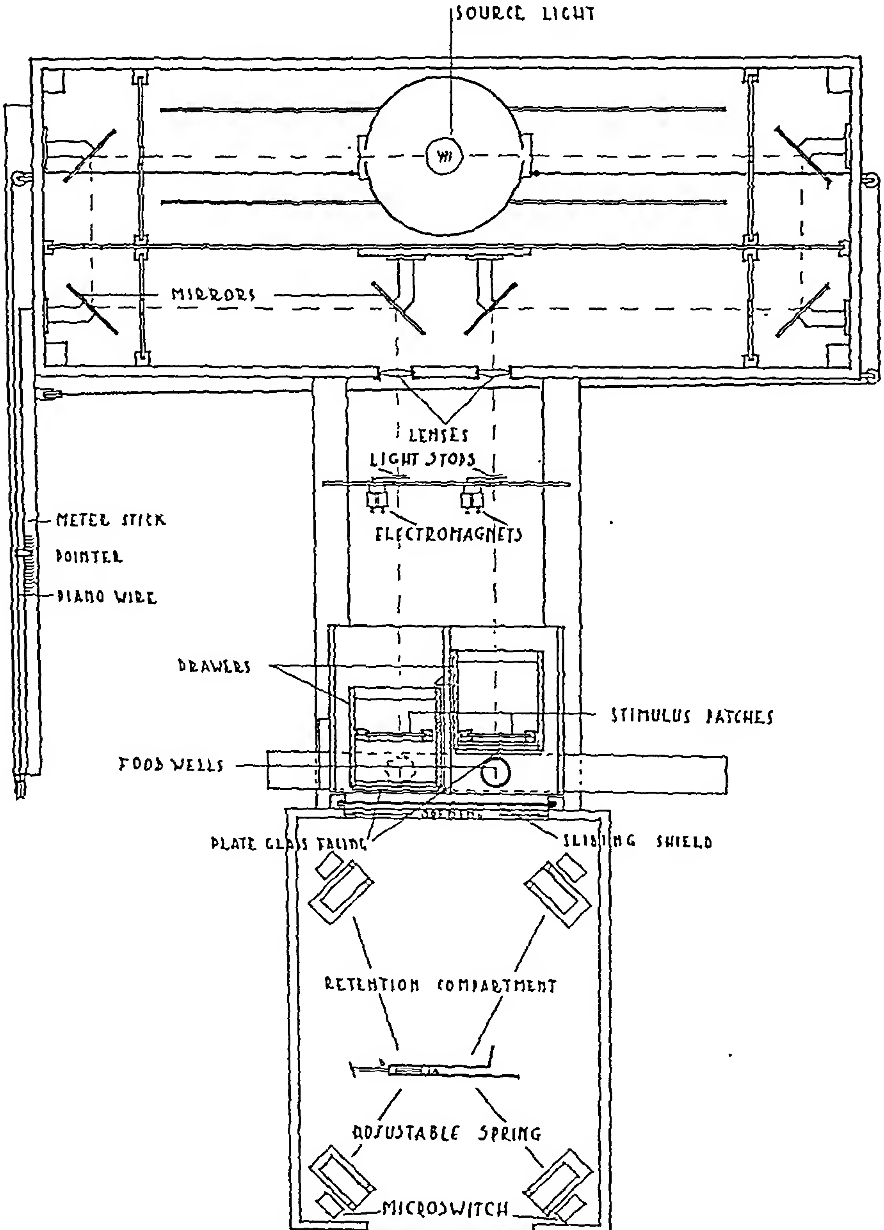

FIG. 1. Floor plan of experimental cage. The adjustable springs which were used in the activity recording apparatus are shown in the insets.

The subject was placed in a metal retention compartment (55 × 75 × 52 cm.). At the front was an opening (Fig. 1) through which it could manipulate the choice mechanism. A sliding wire-mesh screen dropped over this opening in order to prevent the animal from manipulating the choice mechanism until the conclusion of the delay period. The animal

observed the cue stimulus through the screen and through the plate glass facing (15.6 × 12.5 cm.) of a drawer against which it pushed in order to expose a food well. Pushing of one drawer automatically locked the other, so that in case a subject made a wrong response it was prevented from obtaining food immediately after that response.

The stimulus patches (2.5 cm. flashed opal glass squares, 13 cm. apart) were illuminated from behind. Light was reflected from a central source (Westinghouse T-20 1000-watt air-way beacon lamp) to each by a mirror system and lens. The brightness of the stimulus patches as measured with a Macbeth illuminometer was 3.7 millilamberts. Overheating from the lamp was prevented by a current of air generated by a fan whose sound provided a screen for the partial elimination of distracting noises, such as occasional voices from the hallway near by.

At the focal point of each lens the light passed through a small aperture, where it could be conveniently blocked to eliminate the stimulus cues during the delay interval. Blocking was accomplished by a small piece of blackened paper attached to a metal rod. This rod was fastened to the movable coil of a player piano electromagnetic unit. When the coil was energized, it rotated to the horizontal position between the pole pieces of the magnet, pulling the rod upright; when the rod was in such a position the blackened paper was removed from the focal point so that the light was no longer blocked.

Timing was regulated by the experimenter who closed a knife switch to illuminate one of the stimulus patches for approximately 2 seconds.

For presenting interpolated stimuli during the delay period, an overhead light was placed near the center of the ceiling of the retention compartment. The wire-mesh ceiling was overlaid with ground glass. A 50-watt light with metal reflector was suspended above the ground glass, and a piece of milk glass (32 × 26 cm.) was placed immediately below the bulb. A double layer of tissue paper was laid over the glass to increase the diffusion of the light. In order to provide for various intensities of overhead light a General Radiotype 100K Variac transformer was placed in circuit with the light.[2] Snap action microswitches on the control board were used to turn on the light overhead when desired. A click which was barely audible above the sound screen occurred when the switch turned on or off.

Activity recording apparatus. A graphic record of the animal's general activity in the retention compartment was obtained by means of a spring platform recorder. A fiber-board floor covered with galvanized iron was supported at the center by a ball-bearing pivot and in each corner by springs. The type of spring used is shown in the inset and the placement of these springs is shown in the floor plan of Fig. 1. The degree of tension in the spring was variable depending on the distance of block A from block B (see inset). Brass rods attached to blocks A were led outside the cage for the purpose of changing the tension on the springs. Each rod was calibrated, and a stationary pointer mounted alongside the calibrated portion served to indicate the relative degree of tension in the spring.

Beside each spring except the right front a microswitch was placed so that the trigger piece was against the under side of the top floor. The makepoles of each microswitch were wired to a signal marker.

The registration device consisted of a wax paper polygraph. In addition to the three markers for the recording of activity, a fourth marker was activated by a microswitch on the control table to indicate the beginning and end of each trial. The polygraph rollers were driven by a 4 R.P.M. Telechron motor.

In order to keep the activity apparatus approximately equal in sensitivity from day to day, the tension on the springs was regulated so that a given weight at a certain distance from each corner of the cage would close the microswitch.

Comparison of the activity records with the notes of another investigator who observed an animal through a one-way vision screen during trials on the condition of light interpolation served to establish the validity of the apparatus for the purposes of this experiment.

Procedure

After the animals had learned to push against the drawers to obtain food rewards, they were trained in a simple discrimination between light and no

[2] Macbeth illuminometer readings were taken inside the cage at the brightest part of the ground glass ceiling.

light. When an animal had made at least 10 successive correct responses on this condition, a so-called zero interval delay was given; that is, the cue light was turned on for 2 sec., turned off, and the screen was raised immediately. Short delay intervals then followed, and these were progressively lengthened until the animal was performing successfully with intervals of 10 sec. The formal experimental procedure was then instituted.

Condition 1: Non-interpolation. The stimulus light was turned on for 2 sec., the animal was retained in darkness for 10 sec., after which interval the screen was raised permitting choice of right or left by the animal.[3]

Table 1. Pre- and postoperative scores for the criterion trials with the conditions of interpolation and non-interpolation

Subject	Preoperative scores		Postoperative scores	
	Light interpolation	Non-interpolation	Light interpolation	Non-interpolation
	per cent	*per cent*	*per cent*	*per cent*
Mangabey no. 1	83	96	50	81
Macaque no. 7	—	—	46	94

Condition 2: Interpolation of light overhead during delay interval. The stimulus light was on for 2 sec. the animal was retained in darkness for 2.5 sec., the overhead light was turned on for 5 sec., the animal was retained in darkness for 2.5 sec. more, after which interval (10 sec. in all) the screen was raised permitting choice of right or left by the animal.

The criterion which had to be met before training was terminated was a non-improvement one. When 48 trials on each condition had been completed, performance on the first block of 24 trials was compared with that on the second. Testing was discontinued at this time only if the percentage of correct choices on the second was no higher than that on the first block of trials. In the event of improvement in either condition 24 more trials in each condition were given and the second block of trials was compared with the third. Training was continued until the percentage of correct choices on the last was no higher than that on the next-to-last block of trials for each condition. The last 48 training trials which satisfied the criterion are called the criterion trials, and all of these are presented in Table 1.[4]

The daily session was divided into six-trial blocks: interpolation and non-interpolation conditions were given in alternate blocks. Therefore any day-

[3] At the beginning of every session 5 or 6 trials with minimal delay intervals were given; that is, raising of the screen followed immediately after the disappearance of the cue stimulus. These trials were then followed by 3 or 4 more trials whose delay intervals were increased progressively up to the duration to be used in the rest of the session.

[4] Unless a score was higher than 50 per cent it was not considered as improvement even though it was greater than a score of less than 50 per cent made on the preceding 24 trials. And if the score on the first block of 24 trials was 90 per cent or greater, a higher score on the second block of 24 trials was not considered as improvement.

to-day changes in the animal's level of performance affected both conditions equally.

For the presentation of right and left choices a random order left-right sequence was used. This was designed so that an animal with a position habit or simple alternation habit could not score higher than 50 per cent on either experimental condition.

Between trials the monkey remained in darkness for a interval of from 15 to 30 sec. A daily session usually consisted of 24 trials.

Results

Delayed response under standard conditions. Table 1 summarizes the results obtained from the procedure described above. The preoperative score

Table 2. Scores for blocks of trials divided according to the condition of light interpolation and presence or absence of movement during trials (macaque no. 7)

Condition	Brightness of overhead light in millilamberts	Trials with movement			Trials without movement		Reliability of the difference	
		Per cent correct	No. trials with any movement	No. trials with movement to back of cage	Per cent correct	No. trials	S.D.	C.R.
1	0	80	65	28	97	202	.052	3.3
2	.02	73	33	3	91	96	.083	2.2
3	.07–5	63	67	1	82	178	.065	2.9
4	10	25	4	1	48	44	—	—

	Trials with movement	Trials without movement
S.D. diff. between conditions 1 and 3	.077	.031
C.R.	2.2	4.8

of mangabey no. 1 on the light interpolation condition was 83 per cent for the criterion trials. Its performance on the non-interpolation condition was 96 per cent. Both scores were reliably different from 50 per cent (the score which would have been obtained if the animal had been choosing on a chance basis). Quite different results were obtained from this animal following removal of the frontal lobes. The score on the interpolation condition was exactly 50 per cent while that on the non-interpolation condition was 81 per cent for the criterion trials.[5]

These postoperative results were in close agreement with those obtained

[5] For the two sessions in which the first 6 trials were without light interpolation (and the animal began the session with good performance) the score for all 24 trials in darkness was 96 per cent. This is precisely the score which was obtained preoperatively under this condition. On the other hand, beginning the session with poor performance (produced by light interpolations during the first 6 trials) seemed to make performance generally worse during that session.

from macaque no. 7. Its scores on the interpolation and non-interpolation conditions were 46 per cent and 94 per cent respectively.

Effect of activity during the delay period. An analysis of the activity records secured during the postoperative training and testing of macaque no. 7 reveals that although a condition of quiescence is optimal for performance in delayed response, the poorer performance obtained under the conditions of light interpolation cannot be explained in terms of increased activity due to the overhead light stimulus.

Table 2 shows the percentage of correct responses for blocks of trials divided according to the condition of light interpolation and presence or absence of movement during the trials. Absence of movement is defined as absence of any signal marker registration during the delay interval. The table shows that in every case the percentage of correct responses was higher for the non-movement than for the movement trials. It is very important to note, however, that of the 48 trials with the standard conditions of *bright* light interpolation, 44 were non-movement trials. Under this condition of light interpolation the animal failed to score higher than 50 per cent even though it did not move enough during the delay intervals to close any of the microswitches. Total activity during and between trials registered 56 times at the front and 80 times at the back of the cage during the 48 trials, showing that the sensitivity of the apparatus was sufficiently high to record movement when it occurred. On the other hand, a performance very reliably above a chance score (80 per cent in 65 trials) was obtained in darkness trials on which movement occurred during the delay interval. A further indication that the effect of light interpolation in lowering accuracy of performance was due to factors other than increase in activity comes from the fact that the score on non-movement trials without light interpolation was reliably higher than the score on non-movement trials with moderately bright light interpolation.

Almost all the animal's activity during trials was confined to the front of the cage. The number of trials in which movement to the back of the cage occurred is given for each condition in column 5 of Table 2. From these data, there is no indication that the range of activity during trials was increased by light interpolation.

It is strongly indicated that successful performance in delayed response was possible for this operated animal even when it moved to the back of the cage during the delay interval. The score on the trials where movement to the back of the cage occurred (excluding only trials from condition of *bright* light interpolation) was 72 per cent in 32 trials.[6] The probability is only 0.01 that

[6] During these 32 trials the animal chose the right side 23 times, making only 3 errors, while it chose the left side only 9 times, making 6 errors on that side. The animal did not have a right-position habit during the sessions from which these trials with movement occurred: it chose with an accuracy of 82 per cent in 89 trials when the left side was correct. That the animal did not have a general preference for the right side during this period of testing is shown by the fact that in the sessions with bright light interpolation the animal fell into a left-position habit (*i.e.*, it made almost all its errors on the right side).

this high a score would have occurred by chance. The difference between this score and 48 per cent (the score for the 44 non-movement trials with the condition of bright light interpolation) has a high reliability. The probability is only 0.014 that this difference would have resulted by chance.

INDIVIDUAL PROTOCOLS

1. Mangabey no. 1 was a vigorous and active mature male monkey (*Cercocebus torquatus*) which had been under observation for several months before training was undertaken. Prior to coming to the laboratory it had been a pet, and was unusually friendly and well adapted to handling.

Preoperative training. From June to November, 1939, this animal was used in exploratory experiments designed to determine the capacity of a normal animal to perform in the indirect delayed response situation.

The following findings resulted from this work: (i) Adaptation to the indirect delayed response situation was much slower than was that of monkeys used by other investigators in the direct delayed response situation. (ii) After the simple delayed response habit had been established it was very difficult to train the animal to retrieve food (at the back of the retention compartment) during the delay interval without disrupting the delayed response. This habit was finally mastered with delay intervals of 5 seconds or less.

After an interval of approximately six months had elapsed, further training on delayed response was given. The data from the first three sessions are summarized in Table 3. It is important to note in this table that a breakdown did not occur when the light[7] was interpolated. Thus, after an interval of six months without training, this animal was able to perform successfully in both the non-interpolation and interpolation conditions without retraining.

Table 3. Preoperative performance of mangabey no. 1 during first three sessions following a period of six months without training

Condition	Performance
No delay	10 successive correct responses
2.5 sec. delay (non-interpolation)	10 successive correct responses
5 sec. delay (non-interpolation)	1 error followed by 10 successive correct responses
5 sec. delay (2.5 sec. light interpolation)	10 successive correct responses
10 sec. delay (standard procedure as described in section on method)	
Interpolation	86 per cent correct (in 42 trials)
Non-interpolation	89 per cent correct (in 36 trials)

Following this, ten more sessions (387 trials) were given with the standard procedure in order to find the optimal length of session for the criterion trials. On the basis of this further exploration, 24 trials per session were taken as the optimal length. Then four 24-trial sessions were given; and since the scores on the last 24 trials in each condition were no higher than the scores on the preceding 24 trials, the criterion scores were computed from these 48 trials (Table 1).

When all the trials on the standard conditions were thrown together, the score on the non-interpolation condition for this animal was 92 per cent while that on the interpolation condition was 80 per cent. This is a statistically reliable difference, being 4.1 times the standard error of the difference. Although the score on the condition of interpolation was reliably higher than a chance score, it was reliably lower than the score on the condition of non-interpolation. Thus even in the unoperated subject the interpolated light acted to reduce accuracy of performance.

[7] *The brightness of the overhead light used in these tests and in the tests with the standard conditions was 21 millilamberts.*

Operations. The first operation was performed July 22, 1940. Under sodium amytal anesthesia, a large left-sided bone-flap was turned down. The dura was reflected and the extirpation was carried out with the Davis-Bovie electro-surgical unit by first coagulating the veins leading to the sinus. The arcuate sulcus was then incised, and the tissue lying anterior to the arcuate sulcus and its arbitrary extension to the midline was removed.

The second operation, August 5, 1940, was performed on the right side in the same manner.[8]

Postoperative tests. The first postoperative test was undertaken on September 20, 1940. The standard procedure was given, beginning with a block of 6 trials on the non-interpolation condition. Data on the postoperative retention of the habit were obscured by the presence of a strong right position preference. The subject chose the right side six times (3 errors). On the next block of 6 trials (interpolation condition) the position habit was not present. The animal chose the left side three times and made no errors. The position habit reappeared during the next 6 trials on the first condition. On the final block of trials (interpolation condition) the subject persisted in choosing the right side twelve times in succession when the left side was correct. One earlier left-side baiting in this block of trials had been responded to correctly.

Following this test session a number of sessions (totaling approximately 900 trials) were devoted to breaking the position habit and establishing satisfactory performance with a 10-second interval of delay without light interpolation. In the last of these sessions the animal's score was 95.5 per cent in 22 trials. Two trials on the light interpolation condition were also given; these revealed a strong right preference. Through this training period (as before) the correction method was used.[9] That is, when an error occurred the cue stimulus was presented again on the same side and was repeatedly presented on this side until the animal made the correct response. The error and the correction responses were scored together as one trial and one error. During the criterion trials, however, only two correction presentations were given before proceeding to the next trial. This practice was adopted in order to avoid frustrating the animal by a long series of failures in succession and to avoid greatly prolonging the session of 24 trials. The results of the criterion trials are presented in Table 1. A very strong left-sided position preference was present on the "interpolation" trials.

2. Macaque no. 7 was an immature female monkey (*Macaca mulatta*) which had been trained on brightness discrimination by another investigator before it was used in these experiments.

Preoperative training. From December, 1936, to March, 1937, the animal was trained on brightness discrimination. A brightness difference threshold was obtained. Between September and November, 1937, the brightness difference threshold was redetermined. This animal had no training on delayed response prior to operation.

First operation. A one-stage bilateral removal of the rostral part of the frontal association areas was carried out November 15, 1937. The ablation on the left side included the rostral tip of the frontal lobe and all the lateral border as viewed from above. This included the rostral portion of the Brodmann areas 9 and all of 10 and 11 and part of 12. The ablation on the right side was somewhat larger than that on the left. This ablation removed more of area 9 rostral to area 6 than that on the left side. All the frontal tip was removed, but some of the tissue above the olfactory lobe at the base of the skull still remained. All of the lateral portion of the frontal association areas back to area 8 was cleanly extirpated.

First postoperative tests. Between October and December, 1937, the postoperative brightness discrimination difference threshold was determined. The habit was perfectly retained[10] and the difference threshold had not been raised by the operation.

During the period between November, 1938, and August, 1939, a number of exploratory experiments were conducted in order to determine the capacity of this animal for performing successfully in delayed response by the indirect method under different conditions of "extra" stimulus interpolation during the delay interval.

The results of this preliminary work showed that this animal with extensive bilateral

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[8] Both operations were performed by Dr. D. G. Marquis with the writer assisting.

[9] The correction method was not used throughout the training and testing of macaque no. 7.

[10] The score on the retention test with a brightness ratio of 1.4:1 was 80 per cent in 25 trials (beginning 9 days after the operation).

lesions in the frontal association areas could consistently make high scores on delayed response. This was true when the intervals of delay were as long as 28 seconds, providing the interior of the cage was not illuminated with a bright light. Detailed protocols for the work on this animal after the second operation removing the remaining parts of the frontal association areas are given in the next section.

Second operation—August 7, 1939.[11] "Left ablation. A left dural flap was turned back with some difficulty due to adhesions between dura and pia arachnoid. These bled very freely but were not too numerous. Ablation of the remaining tissue—all of areas 8 and 9

Table 4. Performance of macaque no. 7 under different conditions of light interpolation during period between November, 1939, and January, 1940

Session No.	Brightness of overhead light in millilamberts	Per cent correct	No. trials
	1. Whole session technique		
1	0	*94*	19
2	0.02	95	20
3	0.02	75	16
4	0	*100*	20
5	0.02	90	20
6	0.02	75	20
7	0	*95*	20
8	0.02	90	20
9	0.07	95	20
10	0.1	75	20
	2. Split-session technique		
11	0.6	52.5	20
	0	*90*	20
12	0	*90*	20
	0.6	85	20
13	0.6	80	20
	0	*100*	10
14	5	45	20
	0	*90*	20
15	0	*90*	20
	5	60	20
16	5	70	20
	0	*90*	20
	5	73	11
17	0	*100*	20
	5	85	20

—was then carried out, care being taken that no tissue was left on the base of the skull or in the frontal pole. The dura was then closed on this side, and that of the right side opened.

"Right ablation. The dural flap on the right side was not as extensive as that on the left but was sufficient to expose the desired region. On the right side the previous ablation was somewhat larger than that on the left. The present ablation comprised all of area 8 and the remaining tab of area 9 along the midline rostral to area 6."

Second postoperative training. Following the second operation, training was begun September 8, 1939. From the first the animal reacted with a strong right-sided position preference. Thirty-eight sessions were devoted to breaking this position habit and to finding suitable conditions for testing.

[11] The writer is grateful to Dr. M. A. Kennard who performed the operation and was helpful in other respects. These notes are taken from Dr. Kennard's protocols.

During the period between November, 1939, and January, 1940, tests were carried out to determine the effect on performance of interpolation of light from overhead during the interval of delay. The intra-trial conditions were the same as those described in the section on method. After several sessions without light interpolation, ten whole sessions were devoted to either the "interpolation" or "non-interpolation" condition. These sessions were followed by seven sessions where the first 20 trials were with one condition and the last 20 trials with the other.

The results of these sessions are shown in Table 4. Two points should be especially noted: (i) Performance in darkness was consistently high throughout (90 per cent correct, or better) regardless of the just previous performance on the condition of light interpolation. (ii) Performance as high as 85 per cent correct was obtained with the condition of light interpolation when the brightness of the overhead light was as great as 5 millilamberts.

In January, 1940, the standard conditions described in the section on method were used. That is, six trials on one condition were followed by six trials on the other, etc. The brightness of overhead light was 10 millilamberts throughout these sessions. From these sessions the criterion trials reported in Table 1 were taken. A strong right-sided position preference was present on the "interpolation" trials.

Immediately after the completion of the standard tests the same procedure was followed in two more sessions during which the brightness of the interpolated light was reduced to 0.1 millilambert. Thirty-six trials were given on each condition. The scores were 89 and 97 per cent for the conditions of light interpolation and non-interpolation respectively.

Discussion

The results of the present experiment may be summarized briefly as follows: successful performance in delayed response is possible for monkeys after their frontal association areas have been removed bilaterally. The difference between normal and operated monkeys with respect to such performance is not one of presence or absence of the capacity for delayed response,[12] but rather the difference is one of degree of susceptibility to the interfering effects of extraneous stimuli occurring during the delay interval.

Jacobsen's hypothesis that immediate memory is functionally localized in the frontal lobes is not sufficient to account for the results of the present experiment. These results indicate, rather, that there is no real dichotomy between phenomena of memory span and of learning by repeated trials; that is, no dichotomy in the sense that they are two discrete processes, mediated through different neurological mechanisms. In the absence of any such dichotomy the problem becomes one of relating the neurophysiological data concerned with forgetting to present theories of forgetting. The delayed response procedure is very similar to that used in studying retroactive inhibition. The following hypothesis is therefore tentatively advanced: removal of the frontal association areas of the cerebral cortex renders an animal (or human subject) more susceptible to retroactive inhibition.

The absence of a deleterious effect of extraneous stimuli on discrimination learning and retention can be accounted for in terms of the greater degree of learning occurring with a primary rather than with a secondary reward (2, p. 82). McGeoch (16) has shown that the degree of retroactive inhibition

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[12] The results of the present experiment indicate that there is no difference between the effect of frontal lobectomy on the indirect as compared with the direct method of delayed response.

varies inversely with the degree of learning of the original material. The limiting case between delayed response and discrimination learning occurs in Finan's (5) experiment which showed that *one* food reward was sufficient to establish in operated animals a position habit which could be reversed by one food reward on the opposite side. One major difference between "one-trial" learning (delayed response) and "one-reward" learning (Finan's procedure) is the administration of a primary (food) reward in the latter case and the absence of any primary reward during learning in the former case. The effects of extraneous stimuli on discrimination learning may have been insufficient to produce forgetting because the degree of learning with the food reward was great enough to counteract the effects *of those particular uncontrolled interpolations*. In delayed response, on the other hand, the mere sight of food was a less strong reinforcement, and the degree of learning, for this reason, may have been insufficient to withstand the interfering effects of extraneous stimuli.

Further experimentation is necessary in order to determine the validity of this interference hypothesis. Investigations in the field of human memory have shown that the degree of retroactive inhibition is greater when the interpolated activity is similar to the activity accompanying learning than when it is dissimilar. An interpolated stimulus in the delayed response experiment might be expected to have, therefore, a greater effect if it were very similar to the cue stimulus than if it were not.

Melton (17) has suggested that susceptibility to retroactive inhibition as a function of injury to the nervous system should be studied. The results of the present experiment indicate the probable fruitfulness of studies following Melton's suggestion. This problem might be further attacked with procedures other than the delayed response; for example, discrimination learning and conditioning.[13]

Another line of investigation which would have a very direct bearing on the present hypothesis is experimentation with human subjects: the comparison of the degree of retroactive inhibition obtained from a clinical group of frontal lobe patients with that from a control group of normal subjects (and subjects with other than frontal lobe lesions) matched with the experimental group in every respect except presence of brain injury.[14]

In short, the breadth of the present hypothesis demands a broad experimental verification from several lines of study. Since retroactive inhibition is a well-established phenomenon of great importance to systematic psychology, and since it is susceptible to investigation with animal as well as with

[13] Hill (8) has demonstrated "retroactive inhibition" in conditioning.

[14] The use of "psychosurgery" (6) in treating mentally ill patients offers singular opportunity for systematic psychological research. With such patients it would be possible to compare data obtained preoperatively with that obtained postoperatively. As Hunt (p. 162) indicates, however, preoperative psychotic states (such as inattention) may obscure the capacity for memory which the patient actually possesses. But with test materials which have a high attention value, it is possible that valid preoperative data may be obtained from many patients for whom frontal lobotomy has been prescribed.

human subjects, this hypothesis holds promise as a lead to experimentation which will serve to integrate the more precise data from animal experimentation with the data from clinical studies.

Encephalization. That the cerebral cortex should have the major rôle in the mediation of such a behavioral function as memory in the primates is to be expected from the principle of encephalization. Although this principle has had its widest usefulness in the fields of motor functions (3) and sensory functions (14, 15), its application to higher mental functions may prove fruitful. The general statement of this principle is that the central nervous system shows an orderly and progressive development in the phylogenetic series, characterized by the shifting of functions from lower to higher centers and by the increasing dominance of the higher centers in the control of activity.

In the present connection the principle of encephalization would be supported if infraprimates have subcortical centers which perform the same functions as do the frontal association areas in primates. The principle of encephalization could be extended beyond purely sensory and motor functions if frontal lobe removal in the rat or dog and cat had little or no effect on delayed response performance, and if lesions in the thalamo-striate complex produced a marked impairment of memory in these lower animals.

Neuroanatomical data (19, pp. 136–138) suggest that the frontal association areas may have a way station in the thalamus (the nucleus medialis dorsalis) which is analogous to the lateral geniculate body in its relation with the striate cortex of the occipital lobes. The nucleus medialis dorsalis projects only to the frontal association areas (not to the motor and premotor areas) of the cerebral cortex; and this nucleus and the cortical area to which it projects attain their greatest development in the higher primates and man.

Carrying the analogy between the visual system and the "memory system" further, one could look for an analogue of the tectum in the thalamo-striate complex. Such a search would involve a systematic series of lesions in the thalamus and corpus striatum of infraprimate animals with accompanying behavioral experimentation along the lines of the present study.

Summary

After bilateral removal of the frontal association areas, monkeys succeeded in delayed response performance when darkness was maintained during the delay interval. Unlike normal animals, however, the operated animals failed when a bright light was turned on in the cage during the delay interval. The indirect method of delayed response was used throughout the experiment; that is, light—instead of food—was used as the cue stimulus.

These results make necessary the revision of previous hypotheses concerning the functions of the frontal association areas. The hypothesis is suggested that removal of the frontal association areas in primates leads to a marked impairment in their general capacities for memory, because the loss of these areas renders them more susceptible to retroactive inhibition.

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FURTHER STUDY ON TRANSMISSION IN AN ISOLATED NERVE-MUSCLE FIBRE PREPARATION

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Introduction

The properties of the endplate potential (e.p.p.) set up by a nerve impulse at the myoneural junction have been studied in detail in curarized muscle (3, 16). The time course of the e.p.p. in the normal cat's soleus and frog's sartorius, after the spike had been initiated, was investigated by Eccles and Kuffler (6). A study of the eserine effect on neuro-muscular transmission revealed that it was mainly due to a lengthening of the local potentials (e.p.p. +later slow wave) at the junctional region (3). Further, good evidence shows that the e.p.p. is normally responsible for the setting up of the propagating muscle impulse (18).

From all the above investigations it becomes evident that underlying the e.p.p.—or at least part of it—is the transmitter's depolarizing action at the nerve muscle junction. It was therefore important to determine in the single nerve-muscle fibre the duration and intensity of this depolarizing action, causing the e.p.p. Thus the time course of the e.p.p. is determined by two factors: (i) the "active" depolarization caused by the transmitter, whatever its nature may be, comparable in its action to an applied current pulse, (ii) the passive decay of the charge which had been built up. Duration and intensity of the 'active' period (i) was studied in normal and curarized preparations. Also, further observations of the condition of the muscle membrane during the propagating spike have been made. The effect of catelectrotonic potentials, produced by application of cathodal current pulses to the muscle fibres, has been compared with that of the e.p.p. (Section II).

Method

The technique of dissection, stimulation and recording has been described previously (18, 19). When constant current pulses were applied (Section II D) three non-polarizable electrodes were used, one serving as a common stimulating and recording lead. The electrodes consisted of chlorided Ag-wire dipping into a glass tube with a narrow opening and containing saline. A fine thread of cotton wool was pushed through the opening of the glass tube and made a fluid contact with the muscle fibre over a stretch of about 1 mm.

Owing to the ohmic resistance drop in the common path of the recording and stimulating circuits, part of the polarizing potential was superimposed on the electrotonic potential. The polarizing potential thus recorded rises and falls instantaneously with application or withdrawal of the current pulses and therefore can be balanced without difficulty (24).

Results

Section I

During the numerous dissections in the course of these following and earlier experiments some further observations relating to the multiple innervation of muscle fibres have been made. Experimental evidence had already

been obtained for the existence of multiple innervation of practically all muscle fibres in the frog's sartorius (17). These tests when applied to the M. adductor longus gave similar results.

The nerve of the M. adductor longus enters generally at the pelvic end of the muscle and runs distally, giving off small branches. In a few experiments two separate nerve entries, branching from the main nerve and terminating 10–15 mm. apart on one muscle fibre, could be preserved and dissected clear. Thus it was possible to stimulate the isolated muscle fibre through its nerve at two separate points. In some of the experiments below the 'antidromic' stimulus was applied through such a second innervation on the fibre.

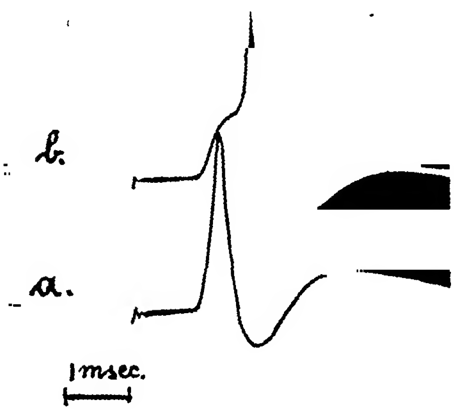


FIG. 1. Potentials recorded in the saline-paraffin interface at the same position at the region of nerve entry. *a*, strong stimulus to nerve *b*, weaker nerve stimulation. The potentials show the same difference in spike latency and in the rise of the e.p.p. component as found when the recording electrode is moved to different positions a small distance apart (18).

In one experiment two different nerve fibres terminated on the same muscle fibre within 1.0 mm. from each other. Alteration of the stimulus strength to the nerve, while recording at its entry into the muscle, revealed a step-like variation in the size of the initial e.p.p. while the latency of the muscle spike itself was altered (Fig. 1). It was suspected that two different nerve fibres of varying threshold, terminating more or less at the same region, were excited separately. This could be proved by moving the recording electrode to different positions around the nerve entry and stimulating with different stimulus strengths. The size of the e.p.p. varied according to the position (18), and also the latency of the muscle spike peak clearly indicated the existence of two separate endplate regions very close together.

In 11 single nerve-muscle preparations altogether similar tests as above were carried out, but only in one experiment (Fig. 1) was there an indication of different nerve fibres innervating closely adjoining regions on the same muscle fibre. The threshold for nerve excitation and the subsequent initiation of a propagated muscle spike remained usually constant within 1–1.5 per cent and further strengthening of the stimulus did not affect the response. It seems likely that in most cases the innervation of an endplate region is supplied by one nerve fibre.

SECTION II

A. *The e.p.p. after initiation of a muscle spike*

If a nerve impulse reaches the neuromuscular junction (n.m.j.) the e.p.p. is set up there and attains a potential as large as the subsequently initiated

muscle spike (18). This is illustrated in Fig. 2. The e.p.p. forms about 90 per cent of the rising part of the action potential, indicating that leading is practically confined to the endplate. After the diphasic trough the potential rises again and reaches 55 per cent of the initial peak potential about 2.3 msec. after the beginning of the e.p.p. The negativity after the main action potential resembles the initial e.p.p. in being largest at the endplate region and diminishing rapidly as the recording electrode is moved away from this region. This, together with other tests (below) clearly indicates that the late potential after the spike represents the further course of the e.p.p. The time course of the intervening phase of the e.p.p. is obscured by the diphasic trough. This trough and the difficulty of estimating the potential time course with interface recordings prevents the accurate estimation of the e.p.p. immediately after a spike had been set up. It can be said, however, that the muscle membrane about 2.3 msec. after the potential has started is much less readily depolarized than normally; a second nerve impulse reaching the endplate at such a time adds only about 10 per cent to the already existing potential.

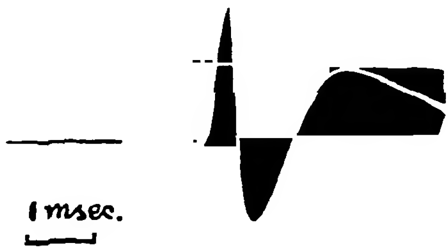


FIG. 2. Interface recording at the endplate region. The potential after the spike rises to 55 per cent total action potential height.

The time course of the e.p.p. in normal muscle can be estimated more accurately from recordings in paraffin oil (18, 19). With this method, however, the contact of the leading electrode cannot be confined to the endplate exclusively and thus the e.p.p. will be diminished relative to the spike, *i.e.* the first phase of the potential will have a greater spike component than Fig. 2. Some allowance has to be made when calculating the true time course of an e.p.p. from such a composite potential.

Two methods can be employed to estimate the time course of the e.p.p. at the endplate.

(i) The action potential set up by a nerve impulse is recorded at the endplate region and a few mm. away from it. A comparison of the two potentials will reveal the additional negativity at the endplate region which is due to the e.p.p.

(ii) Comparison with 'antidromic' muscle impulses can be made. The 'antidromic' impulse is set up by stimulation of the muscle fibre a few mm. from the nerve entry and represents a 'simple' muscle spike without an e.p.p. (19). This 'antidromic' does not differ from a spike due to indirect stimulation (*i.e.* via the nerve) if the recording is done some mm. off the endplate region (cf. Fig. 3B).

A combination of method (i) and (ii) was employed and is illustrated in Fig. 3. Thus Fig. 3A shows an action potential set up by a nerve impulse (N)

at the n.m.j. The negativity following the diphasic wave rises to about 25 per cent of the initial peak potential by 2.2. msec. after the potential start and has almost disappeared in about 7 msec.

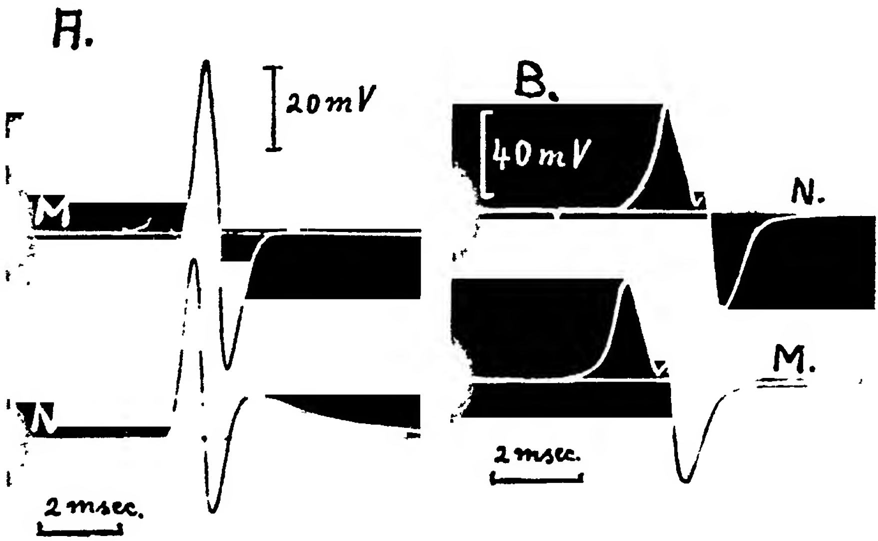


FIG. 3. *A*. Potentials recorded in paraffin at the nerve-muscle junction (n.m.j.). *N* due to nerve stimulation, *M* due to 'antidromic' stimulus. *B*. Potentials 3 mm. from the n.m.j. Note that the two potentials *N* and *M* are nearly identical. (Base line is shown in each record).

The 'antidromic' (M) Fig. 3A was recorded at the same position and no negative wave follows the diphasic phase. It is safe to assume that the main difference between the M and N responses is due to the e.p.p. set up by N.

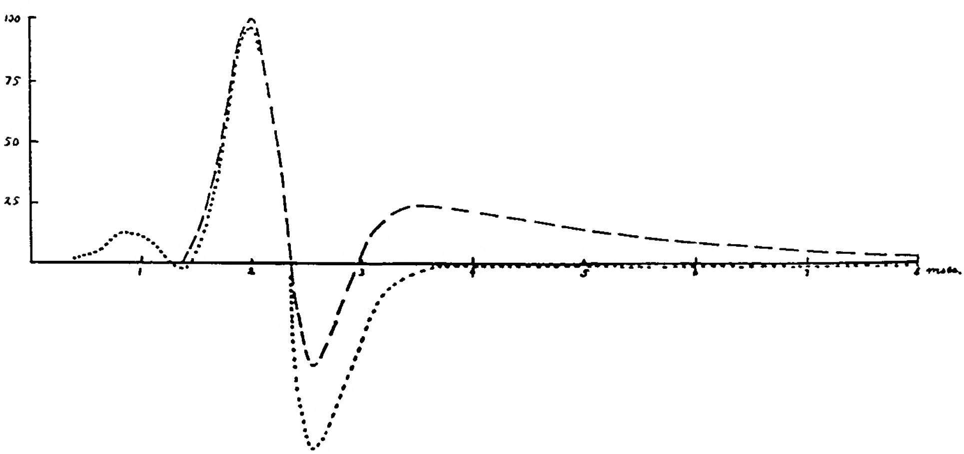


FIG. 4. Potentials from Fig. 3*A* plotted together, with their spike peaks synchronized. The difference due to e.p.p. is well seen.

Besides this, some variations between the two potentials are also expected owing to small lead artifacts which affect M while it propagates towards the endplate region, *e.g.* the small hump before the potential rise was due to a saline blob at the nerve entry. Moreover, the N response is 'growing up' and spreading outwards from the lead, while with M the fully grown impulse propagates past it.

If the M and N responses are recorded 3 mm. from the end-plate region they are practically identical (Fig. 3B). With successive movements of the recording electrode away from the end-plate to this position, all transitional diminutions can be seen in the late negativity of the N response; M on the other hand is not significantly altered.

If M and N are plotted together as in Fig. 4 so that their spike peaks are synchronized the difference between them becomes evident and gives the approximate time course of the e.p.p. following the N spike.

Thus for the normal muscle the approximate e.p.p. time course can be shown before it sets up the muscle spike, (the initial e.p.p., cf. 18) and about 1 msec. later the late part of the e.p.p. can be seen.

B. *Transmitter action as tested by interaction between an 'antidromic' spike and an impulse set up by nerve stimulation.*

The muscle fibre was stimulated some distance away from the endplate region and a muscle spike (M) was set up propagating towards the nerve-muscle junction (19). At different intervals afterwards a nerve impulse was initiated arriving at the endplate synchronously with M or at short intervals later. If it forestalled M, *i.e.* reached the junction earlier, the usual e.p.p. and the consequent muscle impulse was set up and therefore M could not be recorded. The two muscle spikes—one coming towards, the other from the endplate—collided and extinguished each other. If M reached the endplate or was very near to it when the e.p.p. was just rising, the antidromic spike was 'speeded up,' as its 'foot' summed with the developing e.p.p. (cf. also later Fig. 6a, 7b). In Fig. 5 the MN interval is so timed that the transmitting agent in setting up the e.p.p. would start at the middle of the rising phase of M (marked by arrow). The two exposures, the antidromic (M) alone (a) and then M and N together (b), have been super-

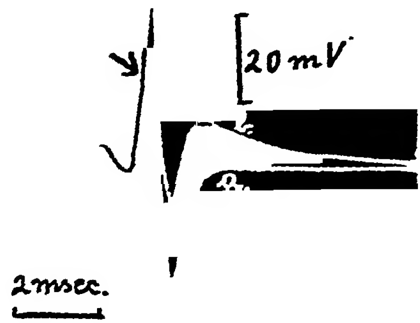


FIG. 5. Interaction between an 'antidromic' muscle impulse and nerve impulse in normal muscle recorded in paraffin. (*a*) Antidromic alone. (*b*) Antidromic + nerve impulse, both exposures superimposed. The action of the nerve impulse starts before the spike peak of *M* (arrow) but does not add a potential for about 0.6 msec. (see test).

imposed and thus the effect of N can be clearly seen as an addition. The nerve impulse arrives at the myoneural junction much too early to set up a muscle impulse after the M spike. There is not even the usual e.p.p. addition as in the early phases of the refractory period (7, 5, 6, 19). During the rising phase (after the arrow), on the spike peak and on the greater part of the falling phase no additional potential can be observed. But eventually a potential is built up, seen as addition to M (Fig. 5). Clearly the depolarizing action of the transmitter gave no recordable potential while the muscle spike (M) was passing the endplate region, and it only became observable late in the decaying phase of the spike. Some inferences can be drawn from the above observation.

(i) during the spike the muscle membrane is fully depolarized, as a nerve impulse, which by itself causes a full depolarization of the membrane, has no appreciable additional effect.

(ii) the depolarizing agent produced by the nerve impulse outlasts the spike and builds up a potential in the refractory muscle. About 2 msec. after the nerve impulse had reached (arrow) the endplate this potential starts to decay.

Thus from the above experiment it can be concluded that the transmitter action lasted *at least* 2 msec. at 28.0°C. Longer durations were observed in other experiments at similar temperatures, and at about 20.0°C. the average duration of transmitter action, calculated as above, lasted 3–4 msec. (cf. effect of temperature on e.p.p., 3). The onset of the decay of the added potential does not imply a cessation of the depolarizing agent. It will be shown in part C, that there is still some transmitter action left during a part of the decaying phase of the curarized e.p.p. A similar action can be assumed to persist after the added potential (Fig. 5) starts to diminish.

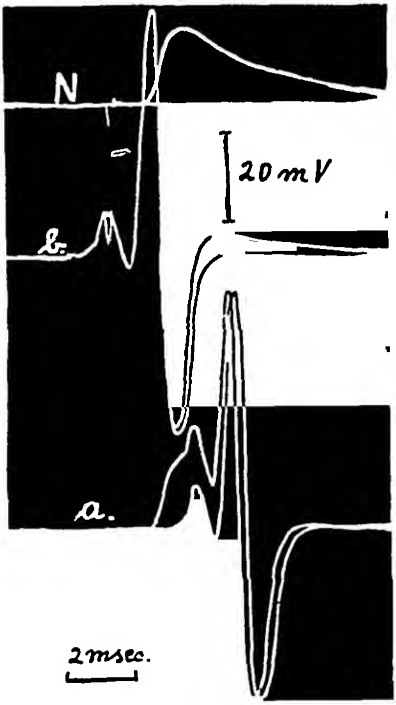
Thus the method above does not take into account the decaying part of the potential addition and therefore the full duration of transmitter action has not been derived. From the quick decay, however, it can be concluded that the intensity of the transmitter action decreases rapidly during that time.

C. *The action of curarine*

A subparalytic dose of curarine has two main effects: (i) it diminishes the e.p.p., (ii) it shortens its duration. After neuromuscular transmission has been blocked an e.p.p. alone is set up. Additional application of curarine only diminishes the e.p.p. still more, without altering its time course, *i.e.* no appreciable further shortening is observed (3, 5, 6, 19).

In the following experiments the same method as in B is applied to completely curarized muscle. The interaction between the antidromic (M) and the pure e.p.p. due to nerve stimulation (N), is illustrated in Fig. 6, 7, 8. The findings were similar to those of Eccles, Katz and Kuffler (3) but could be executed more accurately on the single fibre (cf. discussion). The records were taken with the preparation in paraffin, the curarine action being just paralytic.

If the e.p.p. rise starts synchronously with the arrival of the antidromic it disappears completely during the spike, but appears again, although diminished, after the spike had passed the n.m.j. This shows that the depolarizing action of the transmitter has not ceased by that time. In Fig. 6b the e.p.p. action begins just at the spike peak of M. No addition occurs at



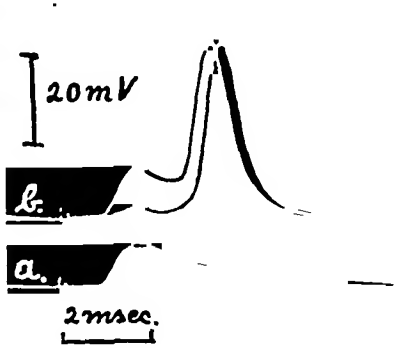


Fig. 7. Action potentials recorded in paraffin. The potential is made monophasic by injuring the fibre near to the recording lead. *a.* e.p.p. due to nerve impulse. *b.* an antidromic muscle spike alone, and then together with the e.p.p. 'Speeding' effect can be observed, e.p.p. disappears during the spike (cf. Fig. 6*a*) and is not built up again after the spike.

Fig. 6. Interaction between 'antidromic' muscle spike and e.p.p. in curarized muscle, recorded in paraffin at the n.m.j. The *N* stimulus in each record is fired at a constant position on the sweep, while the antidromic spike reaches the n.m.j. before (*b*) or after (*a*) the e.p.p. is set up. Top record *N;* e.p.p. due to nerve impulse; (*b*) antidromic alone and together with *N*. *N* adds only a reduced potential (as compared with *N* alone) to the later part of the spike. (*a*) the antidromic spike is 'speeded up' by the e.p.p., which 'collapses' entirely during the spike, as no potential addition is built up after the diphasic wave similar to *b*. The hump in front of the antidromic spike is due to a saline blob at the nerve entry into the muscle fibre, acting as a 'false lead.'

first and then gradually a small potential is built up, reaching about 45 per cent of the control e.p.p. (cf. +0.8 msec. in plottings of Fig. 8). The time course of this addition suggests that a fairly strong action of the transmitter still persists (in the above experiment) about 3 msec. after its start. In Fig. 6a the e.p.p. action begins 1.6 msec. before the antidromic spike (M) has reached the endplate. M is speeded up, *i.e.* although initiated at the same time as previously, reaches the recording electrode earlier (3); but no appreciable further effect on M is seen. The e.p.p. disappeared entirely during the spike of M. The depolarizing agent underlying it could not build up a

imposed and thus the effect of N can be clearly seen as an addition. The nerve impulse arrives at the myoneural junction much too early to set up a muscle impulse after the M spike. There is not even the usual e.p.p. addition as in the early phases of the refractory period (7, 5, 6, 19). During the rising phase (after the arrow), on the spike peak and on the greater part of the falling phase no additional potential can be observed. But eventually a potential is built up, seen as addition to M (Fig. 5). Clearly the depolarizing action of the transmitter gave no recordable potential while the muscle spike (M) was passing the endplate region, and it only became observable late in the decaying phase of the spike. Some inferences can be drawn from the above observation.

(i) during the spike the muscle membrane is fully depolarized, as a nerve impulse, which by itself causes a full depolarization of the membrane, has no appreciable additional effect.

(ii) the depolarizing agent produced by the nerve impulse outlasts the spike and builds up a potential in the refractory muscle. About 2 msec. after the nerve impulse had reached (arrow) the endplate this potential starts to decay.

Thus from the above experiment it can be concluded that the transmitter action lasted *at least* 2 msec. at 28.0°C. Longer durations were observed in other experiments at similar temperatures, and at about 20.0°C. the average duration of transmitter action, calculated as above, lasted 3–4 msec. (cf. effect of temperature on e.p.p., 3). The onset of the decay of the added potential does not imply a cessation of the depolarizing agent. It will be shown in part C, that there is still some transmitter action left during a part of the decaying phase of the curarized e.p.p. A similar action can be assumed to persist after the added potential (Fig. 5) starts to diminish.

Thus the method above does not take into account the decaying part of the potential addition and therefore the full duration of transmitter action has not been derived. From the quick decay, however, it can be concluded that the intensity of the transmitter action decreases rapidly during that time.

C. *The action of curarine*

A subparalytic dose of curarine has two main effects: (i) it diminishes the e.p.p., (ii) it shortens its duration. After neuromuscular transmission has been blocked an e.p.p. alone is set up. Additional application of curarine only diminishes the e.p.p. still more, without altering its time course, *i.e.* no appreciable further shortening is observed (3, 5, 6, 19).

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D. *Application of constant current pulses*

It has already been pointed out (3, 13, 14, 23), that the effect of the local potential at the endplate region is similar to a catelectrotonic potential. It was seen above (B and C) that an e.p.p. applied during the spike has no effect on the greater part of its rising and falling phase. Similar findings were obtained when applying a cathodal current pulse during the spike. If a subthreshold catelectrotonic potential is set up (cf. Method) it rises in an approximately exponential way and after withdrawal of the current pulse it

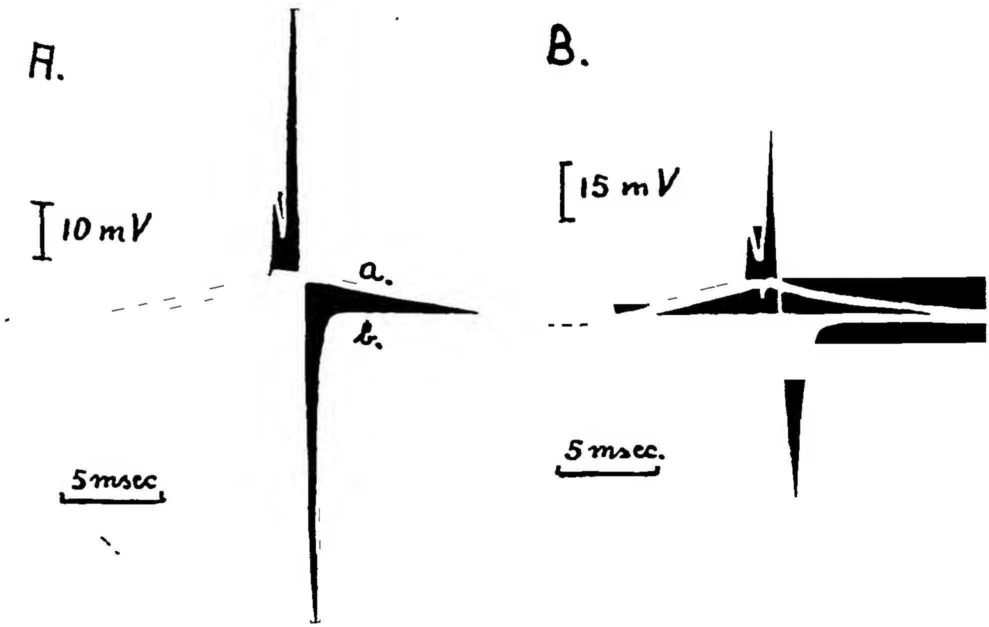

FIG. 9. Recordings in paraffin. *A:* (*a*) just subthreshold catelectrotonic potential, (*b*) the catelectrotonic potential reaches threshold and sets up a spike; the diphasic wave returns to the base line. *B:* a spike initiated some distance away from the recording electrode passes a region where a just subthreshold catelectrotonic potential has been set up. The electrotonic potential 'collapses'. (In *A* and *B* the spike if recorded alone without the catelectrotonic potential shows no difference in height or shape (see text).)

decays similarly (cf. Fig. 9B and also 1, 21, 10, 11, 24, 14, 16). In Fig. 9Aa, a just subthreshold catelectrotonic potential was applied. If the current is withdrawn 0.5 msec. later (Fig. 9Ab) a propagating response is set up (the two exposures are superimposed). After the diphasic wave the potential returns to the base line in the same way as a propagating muscle spike, set up by direct stimulation; there is no addition of potential to the spike which could be attributed to a part of the decaying catelectrotonic potential surviving during or after the spike. It has 'collapsed' during the spike in the same way as the decaying phase of the e.p.p. in Fig. 6a, 7b.

potential similar to Fig. 6b after the antidromic had passed. Evidently, its action had ceased by the time the muscle membrane had recovered sufficiently for a potential to be built up again. Thus no appreciable depolarizing action remains about 4.0 msec. after the beginning of the e.p.p.

The 'speeding' effect and the 'collapse' of part of the decaying phase of the e.p.p. is illustrated more clearly in Fig. 7. The action potential had been made monophasic by injuring the muscle fibre a short didstance from the junctional region. Such an injury spreads quickly and renders the muscle

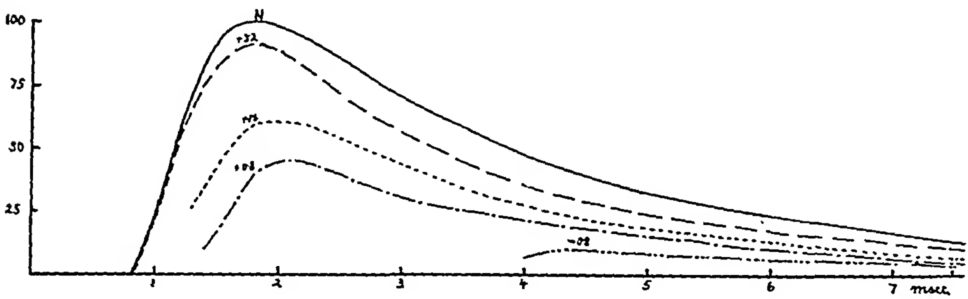

FIG. 8. Plottings of records from experiment partly illustrated in Fig. 6. E.p.p.'s. during or after passage of spike as p.c. of normal e.p.p. (N). E.p.p. starts +0.8, +1.6 or +3.2 msec. *after* spike has reached the endplate region. (note the recovery of e.p.p. size.) If e.p.p. set up −0.8 msec. *before* spike reaches endplate, it collapses during the spike but builds up a reduced potential 3.0–3.5 msec. after its start.

inexcitable in a few minutes. 'Speeding' in excess of 0.2–0.3 msec. has not been observed, presumably as it occurs only during the 'foot' of the spike. Only then can the subsequent breakdown be speeded up by summation of the two subthreshold potentials, *i.e.* foot of the spike and e.p.p. At times the 'speeded' spike was found a few p.c. bigger than the 'antidromic' alone. The plottings of Fig. 8 are from the experiment partly illustrated in Fig. 6, the 'antidromic' reaching the endplate region at different intervals before (+) or after (−) the e.p.p. had been set up (cf. legend).

From the above it can be concluded, that some transmitter action is still left 3.0–3.5 msec. after the e.p.p. had started, as after 'collapsing' it still builds up a diminished potential at such a time.

The method described in this section (C) allows a more accurate estimation of the duration of the transmitter action because the antidromic can be timed to arrive at the endplate also after the e.p.p. had been set up. This was impossible in uncurarized muscle (Section B), for, if the nerve impulse forestalled the antidromic (M), it set up a spike besides the e.p.p. and thus prevented M from reaching the junctional region.

The persistence of the depolarizing agent in curarized muscle is thus found to be about 4 msec.

found. Figure 11 illustrates the electric potential changes at the common stimulating electrode when the muscle was stimulated by short (60μ sec.) condenser discharges. The anodal potential changes increase proportionately to the stimulus strength and decay in an approximately exponential way. The effect at the cathode with a strength of 0.75 threshold differs little from the corresponding anodal potential. With the cathodal stimulus increased to 0.97 (just subthreshold) the potential change is larger and much more pro-

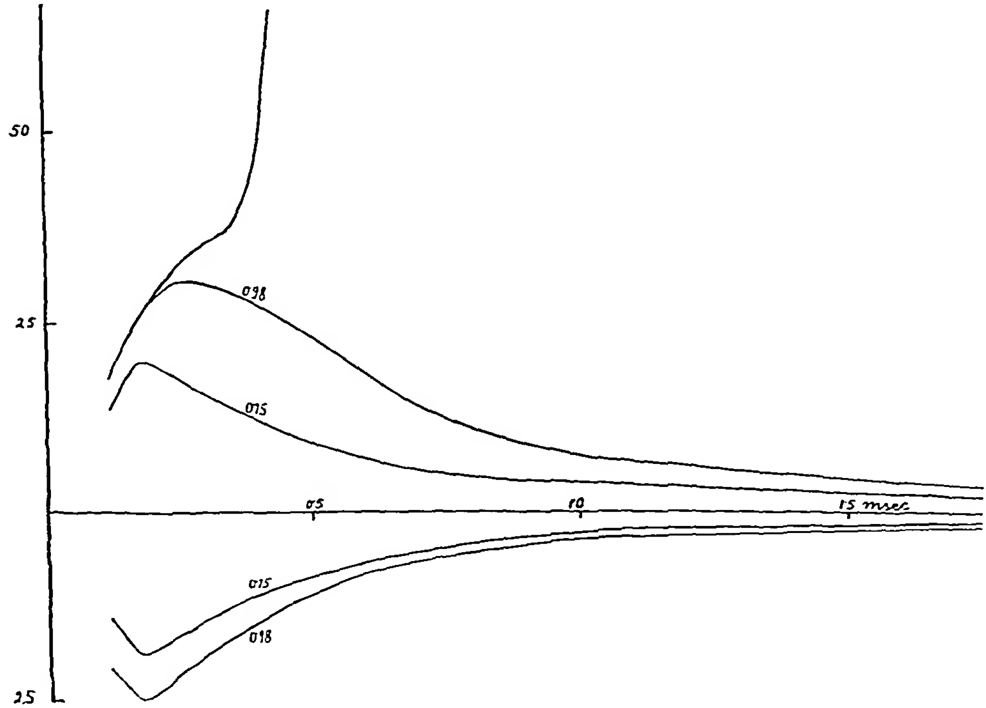

FIG. 11. Potential changes at the stimulating electrode with stimulus strength as indicated. Ordinates: potentials as p.c. of the propagated spike (75 mV).

longed than the corresponding anodal potential. With threshold (1.0) stimulation a fully grown spike of about 75 mV was set up.

From the analogy with nerve (12, 11—Fig. 7, 8, 10; 20) and also from the findings of 'abortive' impulses in refractory muscle (7, 8, 19) these results were to be expected in the normal muscle fibre.

DISCUSSION

The action of a nerve impulse on a muscle fibre may be attributed to a transmitting agent which sets up the endplate potential by actively depolarizing the junctional region of the muscle fibre. In fully curarized muscle the depolarization rises quickly to a maximum which is insufficient to initiate a spike and then slowly decays (*cf.* the pure e.p.p. time course). In curarine-free normal muscle, on the other hand, the depolarization quickly reaches

Similarly a subthreshold catelectrotonic potential is completely abolished by the passage of a spike set up by direct stimulation elsewhere on the fibre (Fig. 9B).

Note the striking difference in the ratio between just subthreshold or threshold catelectrotonic potential and spike size (Fig. 9 and 10). The reason is twofold: (i) in 9A the contact of the common stimulating-recording lead was larger than in 9B, *i.e.*, it recorded the catelectrotonic potential further away from its origin (24), (ii) the threshold value to set up a spike varies after long lasting stimulation or slight damage (11, 20).

The effect of activity diminishing the electrotonic potential set up by an applied current pulse has first been described by Bernstein. (For a full discussion of the subject see Katz, 15, chapt. IV and V.)

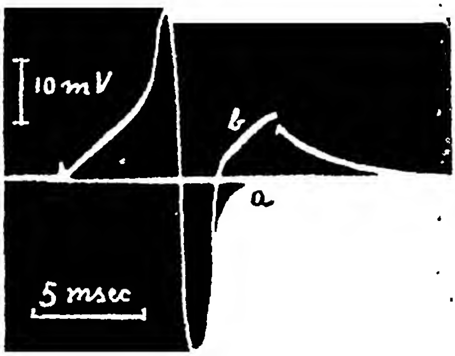

FIG. 10. *a*. The current pulse was withdrawn just when the spike was set up. *b*. The current pulse was continued and a potential is built up after the diphasic wave while the greater part of the spike is not affected. After withdrawal of the current pulse the additional potential decays approximately exponentially. Note the unbalance when current pulse discontinued, shown as an instantaneous potential shift (see text).

In Fig. 10 two exposures have been superimposed. In the first (i) the current pulse was withdrawn immediately after a spike had been initiated. In the second (ii) the current pulse was continued for several msec. The continuation of the current pulse has no effect during the spike itself, but later builds up a potential. This seems to be analogous to the continued depolarizing action of the transmitter producing the e.p.p. in Fig. 5, 6b. The time course of the potential actually built up after the spike by a prolonged current pulse could not be determined because the contraction of the muscle and the consequent changes in resistance between the recording electrodes effect an imbalance of the applied current pulse (cf. Method see p. 309).

When gradually increasing the strength of the constant current pulse there was evidence for local responses when the catelectrotonic potential was near threshold. An inflexion appeared on the catelectrotonic potential, which was not present on the anodal polarization potential when the polarity was reversed. This inflexion was similar to the phenomenon described by Rushton (22) for constant current stimulation of nerve. Owing to the large contact area (about 1 mm. wide) of the non-polarizable electrodes the effective recording occurred some distance from the origin of these local responses which consequently were much diminished in size. When Hodgkins (11) method was used, (brief condenser discharges and fine Pt wires) more clear cut evidence for non-propagating responses in the single muscle fibre was

In curare-free multifibre preparations asynchronism prevented a direct investigation of the effect of an e.p.p. superimposed on the antidromic muscle spike. However, extrapolation of the later phase of such a superimposed e.p.p. suggested that the e.p.p. added about 10 per cent to the spike summit (6, pp. 490–492 and Fig. 5 and 6). But such an extrapolation has now been shown (Sect. II, B and C) to be unjustifiable.

In the curarized multifibre preparation a small addition of e.p.p. was frequently observed on the crest of the antidromic spike. This also was most likely due to asynchronism of the individual spikes, *i.e.* the observed spike peak does not coincide with every individual peak (3, pp. 373–375).

Summary

Experiments were carried out on isolated nerve-muscle fibre preparations from the M adductor longus of the frog (*Hyla aurea*).

1. The transmitter action, *i.e.*, the action of the depolarizing agent producing the endplate-potential (e.p.p.) has been further investigated in curarized and normal muscle. In normal muscle the intensity of the 'active' depolarization decreases rapidly after about 2.5 msec., and has disappeared at about 5 msec. (18–20°C.). These durations are diminished by curarine.

2. During the greater part of the rising and falling phase of a propagating muscle impulse the membrane can not be further depolarized, *i.e.*, no appreciable additional potential can be added during that period by (i) an e.p.p. (ii) a sub- or superthreshold constant current pulse. It is therefore concluded that the polarizability of the muscle membrane is abolished during activity.

3. A propagating muscle impulse approaching the endplate region is speeded up by the e.p.p. in curarized or non-curarized muscle, in agreement with earlier work on the whole muscle.

4. The effects of subthreshold and superthreshold catelectrotonic potenials, produced by constant current pulses, are shown to resemble those due. to the e.p.p. in normal and curarized muscle.

5. Local responses are set up in normal muscle when the stimulus is near threshold.

6. Some findings regarding multiple innervation of the muscle fibres in the M adductor longus are described (Sect. I).

I wish to thank Dr. J. C. Eccles and Dr. B. Katz for their valuable help and advice during the course of this investigation, and also the National Health and Medical Research Council of Australia for equipping and maintaining the workshop in which most of the apparatus was made.

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the threshold value and initiates a spike. The effect of such a depolarizing action, subthreshold or above threshold is imitated to a great extent at the cathode of a constant current pulse, *i.e.* by the catelectrotonic potential (13, 14, 23).

Some of the similarities between the e.p.p. and a catelectrotonic potential are as follows:

1. At a critical potential level a propagating spike is initiated as seen from Fig. 9 and 10 (9, 15, 11). This can be well demonstrated with the e.p.p. in curarized preparations (3, 16) and occurs also in normal muscle adjacent to the endplate (18).

2. During the greater part of the spike the application of a constant current pulse does not add a potential (Fig. 9 and 10), but if it outlasts the spike a potential is built up as the refractoriness passes off (Fig. 10b). Similarly the depolarizing action of the transmitter does not produce a potential during the muscle impulse, but only when outlasting it (Fig. 5, 6b).

3. After cessation of a current pulse the catelectrotonic potential decays passively (Fig. 9). In the e.p.p. of the curarized preparation the cessation of 'active' depolarization is gradual (Sect. II C), so only the latter part of the decaying phase is purely passive.

4. The passively decaying phase of the catelectrotonus is completely abolished by a muscle impulse (Fig. 9A and B). With the e.p.p. a spike gives a similar 'collapse' (Fig. 6a, 7b), but some potential is rebuilt when the impulse acts early in the decaying phase, thus indicating the persistence of some 'active' depolarization (cf. above and Fig. 8, -0.8 msec.).

Additional similarities have also been described (3, 6, 16, 18, 19).

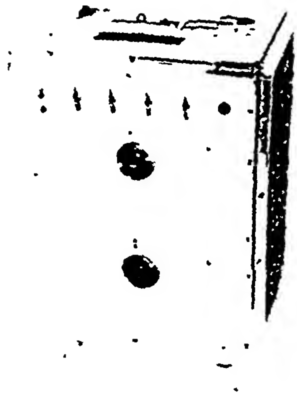
5. The similarity of spatial spread of the e.p.p. and of a catelectrotonic potential are similar.

6. Both prolong the refractory period.

7. The impedance changes accompanying subthreshold current pulses and e.p.p.'s show close resemblance during their decaying phases.

From the experiments showing the building up of the e.p. p. after an antidromic spike (Sect. II B, C) it is possible to determine approximately the time course of the 'active' depolarizing action of the transmitting agent. There appears to be an intense action for 2–3 msec., becoming nearly ineffective in a further 2 msec. No conclusion can be drawn, however, concerning the nature of the transmitting agent. The duration of the depolarizing action was found to vary appreciably in different preparations.

Sections II B, C, D, clearly indicate that during a muscle impulse only the onset of the rising phase can be altered by an e.p.p. (Fig. 6a, 7b). The size and shape of the main part of the following spike potential are unchanged. If there were any readily polarizable membrane in the muscle fibre, which is not affected by an impulse, some additional potential would be added with applied current pulses (Fig. 9 and 10). Thus practically the whole polarizability of the muscle fibre must be confined to the excitable membrane which 'breaks-down' during activity (2; also earlier findings discussed by Katz, 5).



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CHANGES IN NORMAL ELECTROENCEPHALOGRAM OF *MACACA MULATTA* WITH GROWTH*

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INTRODUCTION

THE CORTICAL POTENTIALS of the human infant have been shown by a number of investigators (1, 2, 3, 6, 7, 8, 9, 10) to change with increasing age. In the present investigation similar changes were found in the electroencephalogram (EEG) of the infant monkey (*Macaca mulatta*). The problem was undertaken at this time because the EEG of monkeys was known to resemble that of man in many of its major characteristics, and it was felt that analysis of such cortical potentials might be of value in further interpreting the functions of the central nervous system. Since the period from birth to maturity in the macaque occurs in the relatively short space of four years, the age of the animal and the expected pattern at a given age are significant in the analysis of normal and abnormal EEGs.

METHOD

A Grass, 3-channel ink-writing oscillograph was used.† Solder electrodes 4 mm. in diameter moistened with electrode paste were attached to the shaved skin of the head with collodion. Six positions were used—left and right frontal, parietal and occipital areas (Fig. 1). Indifferent electrodes were attached to each ear (E). Records were usually made in four arrangements of electrodes which were supplemented by others when indicated. The usual recordings were (Fig. 1): (1) Direct leads from the left side of the cortex to Ear, 1E, 2E, 3E. (2) The same from the right, 4E, 5E, 6E. (3) Interhemisphere, 1–4, 2–5, 3–6. (4) Inter- and intra-hemisphere, 1–3, 2–5, 4–6.

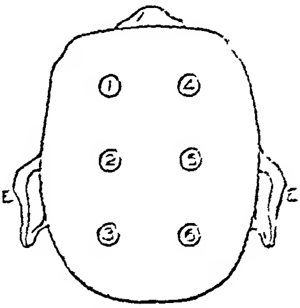

FIG. 1. Diagram of monkey's head showing position and numbers of scalp (1–6) and ear (E) electrodes as used throughout the experiments.

The larger monkeys were restrained in holding boxes (Fig. 2) but the smaller infants were swaddled and held by one of the investigators throughout the procedure. The eyes were bandaged shut and the room kept in semidarkness. No anesthesia was used, but after preliminary struggling and some periodic effort the monkeys were usually sufficiently quiet so that cortical potentials could be recorded without contamination by muscle potentials. One hundred and fourteen electroencephalographic records were taken from 34 monkeys. Seventy of these were from 17 animals of known age during the first year and a half of life. The remaining 44 records were from 17 animals of known weight but the ages of which were only estimated from the size, weight and state of maturity of the individual.

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† EEG apparatus was made possible by the courtesy of a grant from the Josiah Macy Jr. Foundation to the Department of Neuroanatomy, Yale University School of Medicine.

as the measurable variables, rate and amplitude. The effect of sleep, of opening and closing the eyes, and of stimuli such as noise or light on these potential changes can be seen and accurately interpreted. Amplitude varied at different ages from 20 to about 80μV. Three frequencies, or rates per second, can be in general separated, called usually fast, medium and slow, or beta, alpha and delta (1) when used to characterize the pattern in man.

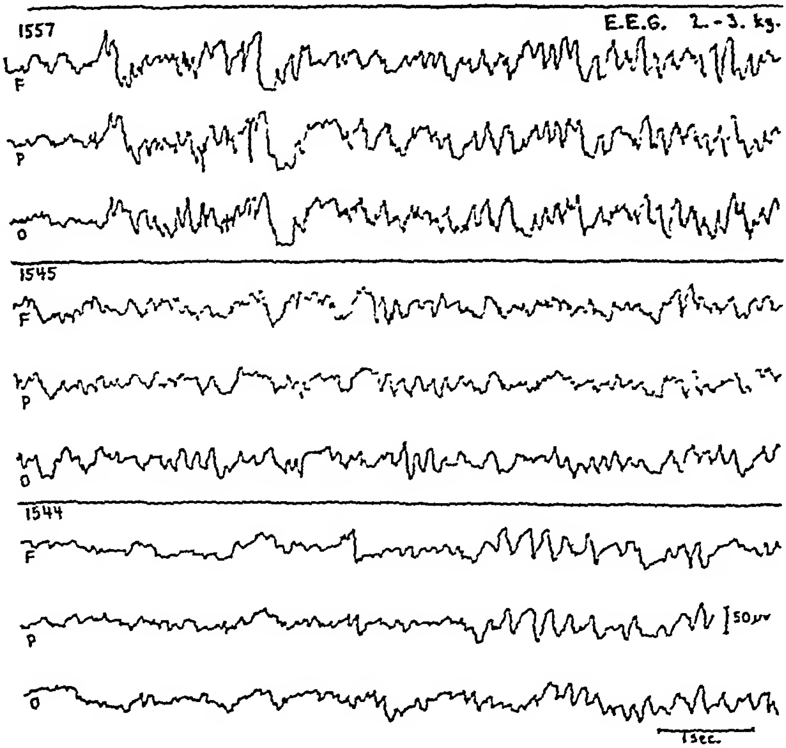

FIG. 3. Normal EEG records from 3 *Macaca mulatta* of 2–3 kg. weight from F, frontal, P, parietal, O, occipital leads. Indifferent electrodes on ear.

The most obvious waves in both man and monkey are of medium frequency varying with age in the monkey from 3–4 to 10–12 per sec.

No focal changes were noted with growth, the oscillations of potential were usually of the same rate, amplitude and direction (+ or −) at the same time within one hemisphere from frontal parietal or occipital areas. Furthermore, the intra-hemisphere oscillations were usually alike in all respects on the two sides. But potentials from interhemisphere leads were usually lower than those of intra-hemisphere recordings made simultaneously. This difference was found to be accentuated following cerebral cortical ablations (4).

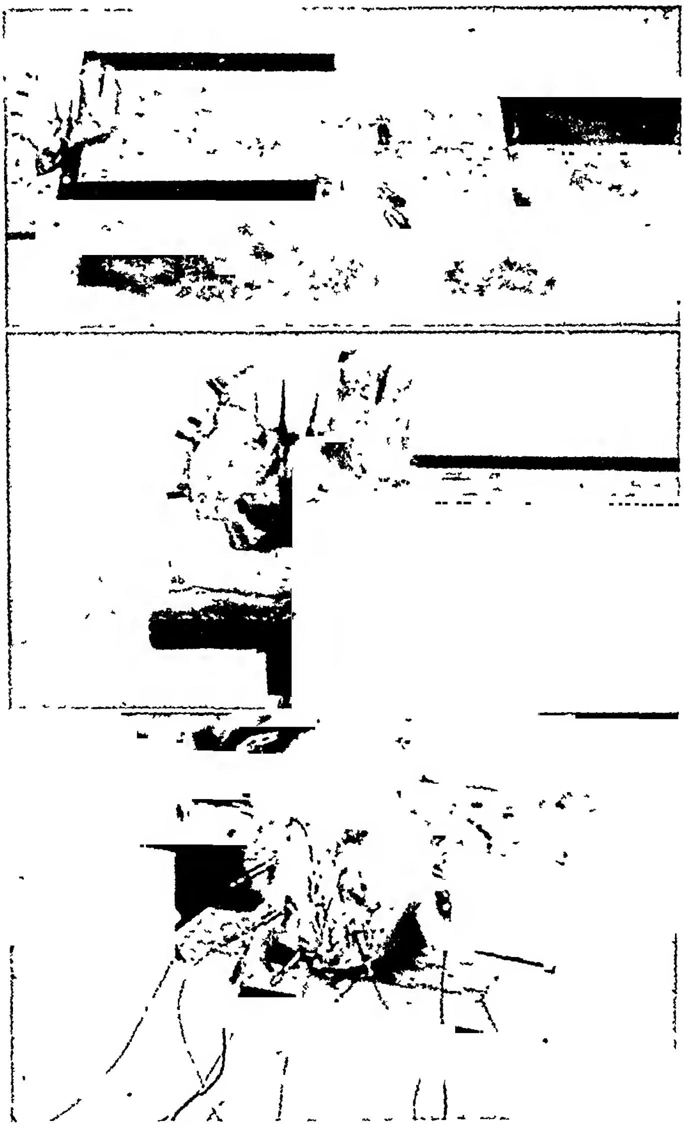

FIG. 2. Holding box for monkey. Solder electrodes and leads in place for recording EEG. The animal is conscious and in no pain.

DATA

General characteristics of normal EEG. At any age the EEG of the normal monkey has a definite pattern and characteristic features which resemble those seen in the human (Fig. 3). Although there are individual modifications of pattern and changes with age as will be shown, these can be expressed

There was an individual difference in the amplitude of the potential changes in these infants under a week old, as shown in eighteen records made on 11 infants. It appeared also in 7 records made on 7 infants on the first day of life. When the records were arranged according to the weight of the infants it was found that smaller infants showed simpler records with slower amplitude than larger. Body weights varied from 355 to 515 gm. at birth. No data were available concerning exact number of days of gestation. It is possible, however, that the smaller infants were actually younger than the larger, and functionally better developed animals. Since the differences at birth were so striking and the character of all records changed most markedly during the first two or three weeks, it may be assumed that in the infant monkey relatively rapid cortical development occurs during the immediate pre- and post-natal period.

Localization of function within the various cortical regions has been found in the human infant (8) in which the region about the central sulcus first shows changes in potential. In the monkey, although this area is known to develop anatomically and functionally before either frontal or occipital poles, no very significant localization of activity appeared on records made from the surface of the head, although in a few instances the occipital leads registered less activity than did either frontal or central. It is probable that this is due to spread of current on the very small surface of the infant head, which is never larger than 6 cm. in diameter, and on which the edges of the electrodes could never be farther than about 1 cm. apart.

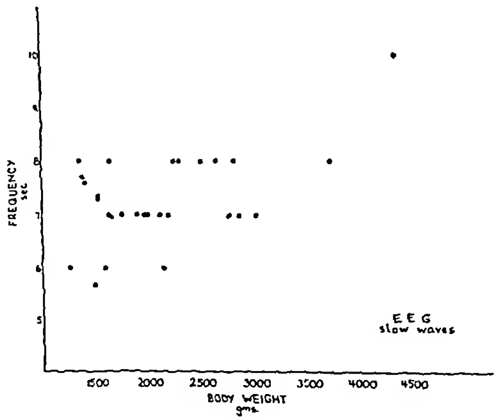

FIG. 5. Frequency of EEG waves in young and adult *Macaca mulatta* showing range of 7–10 per sec. after 2nd year (weight 2500 gm.) of life.

EEGs at four weeks. By the fourth week there were very noticeable changes in the records. Amplitude increased slightly and rate changed from about 2–3 to 4–5 per sec. (Fig. 5). The records also became more complex and less variable than those of the new-born. The wavering base-line producing slow waves of 2–3 sec. frequency was less often present. Such changes appeared occasionally in the records of the two-week-old infants, in others slightly later. In all, the most distinct and rapid changes which appeared at any time took place before the end of the first month.

At this time variability still persisted and a type of wave which gradually disappeared during the latter part of the first six months was very

During sleep, the regularity of pattern became disturbed and the amplitude increased so that large irregular oscillations would appear gradually in all leads and disappear suddenly if the animal were wakened. If records were taken from a monkey awake and unblindfolded but in the dark, there would be a characteristic "damping" of the picture when lights were turned on. Amplitudes became smaller and regularity of pattern increased. Loud sounds or focussing of the attention by any other means caused the same type of change.

EEG during first week of life. The EEG of the new-born infant monkey can be easily identified by its exceedingly low and slow potentials, and by

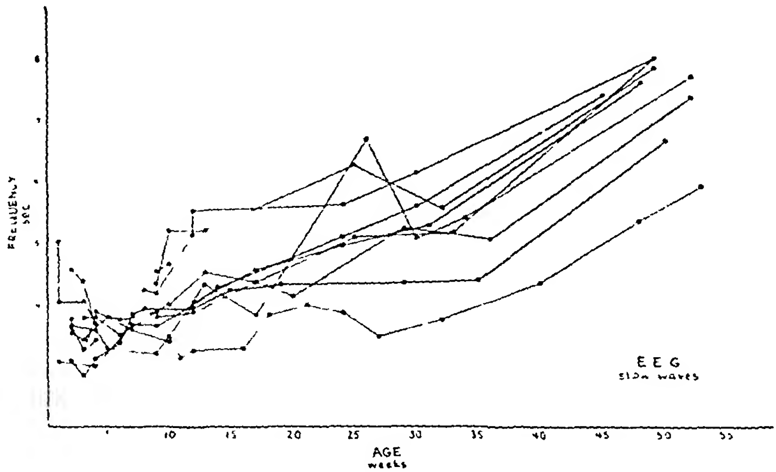

FIG. 4. Frequency of EEG waves during 1st year of life. Lines represent individual animals (*Macaca mulatta*).

the simplicity of pattern (Fig. 4) as compared with that of older animals (Fig. 3). At most there is relatively little activity but what is present varies markedly, showing, at intervals, bursts of 2–3 per sec. waves. The base-line is unstable because of long slow waves, each one lasting 3–6 seconds. There are relatively few fast oscillations.

At this age it was found impossible to correlate the potentials with sleep because the infants, during the first two months are either asleep, drowsy or struggling. When held for the EEG recording, they usually vocalized and struggled, then quickly relaxed and clung limply with eyes shut. During this time, when respirations were slow, deep and regular, it was assumed that the infant slept, but the stages in relaxation were so slight and gradual that no true state of sleeping was ever certain. In the older animals, sleep could at times be definitely ascertained. From the evidence of Smith (11) it is probable that the variability in activity of the cortex in the new-born was in part at least dependent on the state of sleep or wakefulness.

Sleep could not be distinguished from waking though there were intermediate stages. The flattening of the waves which appeared with awakening could be made more pronounced by external stimuli such as loud sound, opening of the eyes or a bright light. Mere focussing of the attention to noise, etc., seemed to produce this flattening of the waves and decrease in amplitude.

EEGs of monkeys weighing from 2000–2500 gm. The largest number of older monkeys on which records were made lay in this group (17 records on 8 animals) which consisted of animals probably in the middle of the second year of life. Because the rate had now increased to about 8 per sec. and amplitude was decreased, many of the smaller variations seemed to be ironed out (Fig. 3). These records were not now very different from those of the adults.

EEGs above 2500 gm. weight. All the records from older animals (14 records on 7 monkeys) were characterized by low amplitude and rapid rate which usually reached 10, and occasionally 12 per sec. was comparable with the alpha rhythm of the human. These records were very uniform but different animals showed individual characteristics.

The reason for the marked decrease in amplitude of the waves was possibly, as in man, due to increase in thickness of the skull. This has been found to materially affect size of recorded potentials. But, records in a few instances were made directly from the surface of the brain of infants and of adults and in these cases also the character of the two ages remained different. Those of the infant showed widely variable potential changes with great fluctuation in amplitude and rate, while those of the adults had more even and regular fluctuations with more rapid rate and lower amplitude.

Discussion

From these data it seems that the evolution of the adult EEG in the young monkey has much in common with similar changes which occur during the same process in man. In each, the picture becomes stabilized to the adult level at a relatively early age; in man at 12 years and in the monkey during the second year; well before maturity of the entire organism. In each, the most pronounced changes occur very early; in man during the first year and in the monkey during the first month. In both the changes with age are those of increase in frequency of potential waves and of an early increase in amplitude followed by a later and slighter decrease in the latter.

The changes in EEG have been correlated in man with the anatomical and physiological development of the central nervous system and may be so correlated in the monkey. Lindsley (7), quoting Weinbach, says that cortical potential changes can be correlated with growth of brain in man. This correlation is about as good in the infant monkey for in these animals, weight of brain increases largely during the first month and little after the second year of life (5).

noticeable. This was seen as infrequent bursts of very rapid waves, occurring spontaneously and not related to any known state of the animal. They lasted only 2–3 sec. at a time and the rate was about 15–20 per sec. They were not unlike the episodes which we have observed after cerebral ablations in older animals (4). At this age sleep and waking could sometimes be distinguished. During sleep the medium frequency waves were accentuated.

EEGs from 2d to 6th month. By the second month amplitude of the waves had increased greatly, often to the maximum for the particular animal. There was by then no difference in occipital, frontal or parietal records. Amplitude by the fourth month was always maximal and it then often decreased slightly during the remainder of the first six months of life. Frequency increased (Fig. 6) to a rate of 5–6 per sec. by the fourth month and as high as 6–7 per sec. by the sixth month.

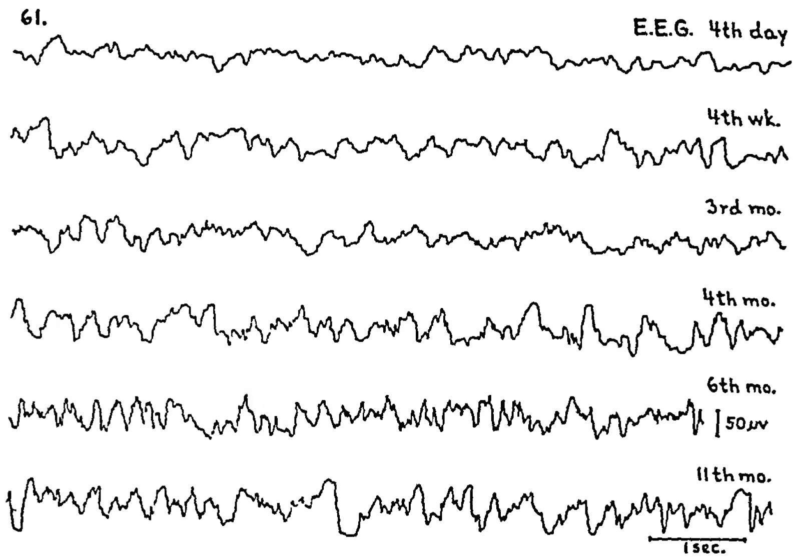

FIG. 6. EEG records at intervals during 1st year of life of a *Macaca mulatta*. With increasing age amplitude, rate and complexity of pattern are increased.

EEGs from 6th to 12th month. During the second half of the first year of life records were taken monthly from two animals of known age and from four others of about the same weight (1700–2000 gm.). In this age group frequencies of the most obvious waves had increased to between 6 and 8 per sec. (Fig. 6). Amplitudes, in contrast, had decreased. Throughout the record of one individual on a single day or for many days, there was less variability in the type of potential changes than in the younger infants. The wavering base-line had almost entirely disappeared but the medium and fast rhythms formed complexes as intricate as those seen in the adult animals.

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The development of motor function in the monkey also occurs largely during the period of most marked change in EEG, *i.e.* the first two months of life. During this time the infant monkeys change from a state in which they are able only to right, climb and grasp reflexly to one in which there are responses to visual and auditory stimuli and in which walking, climbing and the beginnings of voluntary prehension appear. By the end of the first 6 months infants are capable of an adult type of motor performance. All evidence therefore points to the fact that with the development of cortical integration of motor power there is a coincident change in electrical potentials and that this occurs at the time of maximal anatomical and functional differentiation of the cerebral cortex.

The electroencephalogram of the adult monkey has been shown to be similar in many characteristics to that of man. That there is not, however, as much localization of function within different areas of the cortex might be expected because of the relatively small size of the monkey brain, and of the relative lack of complexity in its organization.

Conclusions

1. In the infant monkey cortical potentials (EEG) begin to develop at or before birth, but are not well demarcated until three or four weeks after birth.
2. From this time until the end of the sixth month there is progressive development and elaboration of the EEG until it resembles that of the adult.
3. Frequency of waves is about 2–3 per sec. immediately after birth. It increases to about 7–8 per sec. during the first six months and then slowly to about 10–12 per sec. by the end of the second year.
4. Amplitude is low at birth, increases during the first six months and then slightly decreases.
5. During the growth period the EEG becomes at once more complex and more uniform. There are less variations of base-line and of type of potential.
6. The effect of sleep can be detected in the older animals, but in the newborn infant fluctuations in state of waking or sleeping are too slight and gradual to be correlated with the marked fluctuations in type of EEG which may however be related.
7. The changes in the development of the infant monkey are like those described for man and are as far as is known coincident with the anatomical and functional development of the cerebral cortex.

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EFFECT ON ELECTROENCEPHALOGRAM OF LESIONS OF CEREBRAL CORTEX AND BASAL GANGLIA IN *MACACA MULATTA**

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INTRODUCTION

WITHIN the last five years the electroencephalogram (EEG) has become recognized as a useful means of diagnosis of specific lesions in the central nervous system in man. Abnormal foci such as tumors (23, 21) can often be detected and the changes which result from epilepsy (6, 7, 8, 13, 21) or from increased intracranial pressure (5) are now easily recognized. The monkey, under normal circumstances has an EEG which is comparable in many respects to that of man (17). In the present article the effects on EEG of focal lesions of the central nervous system of such animals are described.

METHOD

Lesions have been made in various portions of the *cerebral cortex* and *basal ganglia* of 41 monkeys (*Macaca mulatta* or *Cercocebus torquatus atys*) and EEGs taken before and after operation have been compared. Both acute and chronic experiments were carried out. In each, the lesions were made by the same operator and the same technique. The acute experiments were executed under dial anesthesia (0.3 mg. per kg. given, ½ intraperitoneally and ½ intramuscularly) which ensured a long and even state of anesthesia. Operations for the chronic preparations were done under sodium amytal and with aseptic precautions. Except in the acute experiments, records were made on unanesthetized animals restrained as shown in the preceding article (17). In nearly every instance, after a certain amount of struggling the animals relaxed and permitted the recording of cortical potentials uncontaminated by those of muscular activity. The subjects were blindfold and in semidarkness in a quiet room.

A Grass, three-channel, ink-writing oscillograph was used.† Six solder electrodes were applied with collodion to the shaved skin of the head, over the frontal, parietal, and occipital regions in the six positions described in text and figures of preceding article (17). The same combinations of electrodes were also routinely recorded and supplemented by others whenever indicated.

EEG records were made on chronic preparations of two types, namely, those operated on in early infancy and older animals. The two groups will be discussed separately as results show that, as is known in human cases, the effect on the EEG of lesions sustained before either anatomical or physiological development is complete may be quite different from that on the EEG of a mature brain.

DATA

A study of the preoperative records of the monkeys of this series shows normal EEG patterns such as have been described previously. The varia-

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† EEG apparatus was made possible by the courtesy of a grant from the Josiah Macy Jr. Foundation to the Department of Neuroanatomy, Yale University School of Medicine.

and smooth as compared to the more variable and rapid oscillations in the preoperative picture (Fig. 1).

Occasionally such changes did not appear on the first day after operation, but on the second, and became more pronounced on the third and fourth, gradually diminishing after that date. The time sequence follows closely that previously observed on a large series of animals of the reaction of tissue to injury. Cerebral edema is usually worst on the second postoperative day,

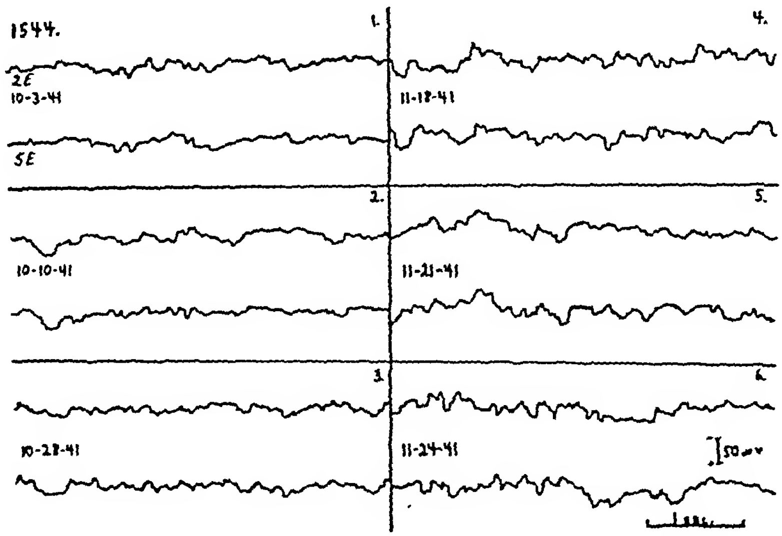

Fig. 1. Record from a large Macaca mulatta (3–4 kg.): 1, normal; 2, After "control" reflection of skin and bone flap only on Oct. 6, 1941; 3, and 4, After ablation of left areas 4 and 6 on Oct. 27, 1941; 5, and 6, After removal of entire left hemisphere on Nov. 19, 1941. Records are from two sides of head (2E left and 5E right parietal).

and severe only for three to four days. It may subside during the ensuing week.

B. Focal effects of cortical lesions. During this series of experiments, pre- and postoperative EEG records have been made from monkeys with bilateral and unilateral lesions of every area of the cerebral cortex. The majority have had lesions of the sensorimotor cortex, in particular of the motor areas in the frontal lobe. In many cases these were combined with subcortical lesions in the caudate and putamen.

1. Hemispherectomy (4 monkeys). When the entire cerebral cortex was removed from one hemisphere leaving basal ganglia uninjured, no characteristic changes in EEG (Fig. 1) pattern resulted. After the initial non-specific changes, the picture returned to that which preceded operation, and in two such instances, has remained so for more than three years after operation. The other two animals which were kept alive for shorter periods likewise showed no changes. Leads placed in the usual positions recorded patterns which were identical for the two halves of the skull, except that those lying

tions due to age, sleep, light, sound or other factors of attention, or to individual peculiarities could be recognized, and hence, new characteristics appearing after cerebral ablations could be detected.

CONTROLS

The effects on EEG of elevation of skin, bone and dural flaps was tried on three animals. In the first, the skin flap alone was opened and closed, in the second, skin and bone flap, and in the third, the dural flap was opened and the cortex left exposed to the air for ten minutes but without being touched. EEGs measured on the days following these procedures showed no change in rate and none in pattern except that the amplitude was slightly diminished for a day or two at most. It was thought that this latter was due to scalp edema which increased the distance between the active cortex and the recording electrodes. Later, cortical lesions in these same animals produced the characteristic changes in potentials described below.

ACUTE EXPERIMENTS

Under dial anesthesia and with leads placed directly on various areas of cortex, records were taken from three animals. No effect on EEG was observed from any lesion made either in cortex or basal ganglia by this means. In two cases portions of cortex were removed by suction successively from areas 4, 6, and the postcentral gyrus, followed by lesions in caudate and putamen. In one of these the frontal and occipital lobes were later extirpated. No effect on EEG developed as recorded from islands of tissue left intact in area 4.

The third animal's cortex was left undisturbed and the caudate was removed via corpus callosum first from one and then both sides. Later, the putamen was destroyed through a small hole in area 8. There was no change in EEG. As will be discussed later, similar lesions in chronic preparations caused marked changes in EEG. The lack of such changes here can be attributed either to the acute state of the preparation or to the anesthetic.

CHRONIC EXPERIMENTS

A. Non-specific effects of lesions of central nervous system occurring during first postoperative weeks. Immediately following injury to the central nervous system, *i.e.*, during the first one to ten days, EEGs were altered materially and in the same way no matter where the cerebral lesion had been made. The size of the lesion, however, affected the duration of these non-specific changes, as small lesions produced effects lasting only a day or two, while the effects of larger ablations might be visible for more than a week.

The records during such a period as compared with those preceding operation were lower in amplitude and slower in rate. The changes in amplitude, as in the controls, could be due to scalp edema, but, in addition, the rate of the 8 per sec. waves was *often* slowed as much as 2–3 cycles and *usually* 1–2 cycles per sec. Moreover, during this period, the fast component became less obvious so that the entire record appeared even, regular, slow

2. *Lesions of sensorimotor cortex (9 monkeys).* Neither unilateral nor bilateral ablations from any part or parts of the sensorimotor cortex produced any very significant change in EEG in the older animals (Fig. 2). It was noted several times that the postoperative records showed a greater tendency to bursts of deep regular 8 per sec. waves called hypersynchrony by Jasper (11, 13), but reexamination of all records did not show that there was anything more than a suggestion that these irregularities were more frequent after than before operation. In the infant records, as will be seen later, this tendency became much more definite.

Unilateral and bilateral ablations were made as follows: Area 6, two monkeys; area 4, one monkey; areas 4 and 6, four monkeys; postcentral gyrus, two monkeys; and angular gyrus, two monkeys. Each lesion was followed by the expected clinical picture characteristic for the areas injured. In some instances records have been taken at intervals for as long as two years after operation.

3. *Lesions of frontal association areas (4 monkeys).* Pre- and postoperative records from 4 animals which had had extirpations of areas 8–12, the cortex lying rostral to the motor areas, showed no significant changes in pattern and no localization of lesion. These animals after bilateral ablations developed the changes in behavior and greatly increased activity which are characteristic of removal of these areas and which are considered by some to indicate "release of function" of one type just as spasticity is assumed to be another evidence of a release mechanism. In neither instance did EEGs show release of function.

4. *Lesions of temporal and occipital lobes (5 monkeys).* Postoperative records after bilateral occipital lobectomy in one animal (made two years previously), revealed nothing unusual except that there was no response in EEG to visual stimuli of light and dark. The monkey had, clinically, no vision but had shown response of pupillary constriction to light. Records were made on four animals before and after temporal lobectomies. There were no changes in the character of the EEG.

5. *Cortical ablations made in infancy (8 monkeys).* As reported previously (17) EEGs of normal infant monkeys have been made from birth until 2 years of age. Seventeen such infants had ablations from the central nervous system during early infancy. Eight of these were in cortex only and with 3 exceptions these eight, like the older animals showed no change in EEG after the first generalized reaction.

The three exceptions were monkeys which had had all of areas 4 and 6 removed bilaterally. After the initial slowing and flattening phase of the waves the pattern returned to normal for some months. In each case however, there *later* developed a slight but definite change which was like that found following lesions of cortex and basal ganglia combined, the characteristics of which will be described below. There are two possible explanations for this; either large cortical lesions of areas 4 and 6 made in infancy will eventually produce this alteration by rearrangement of cortex itself, or, as

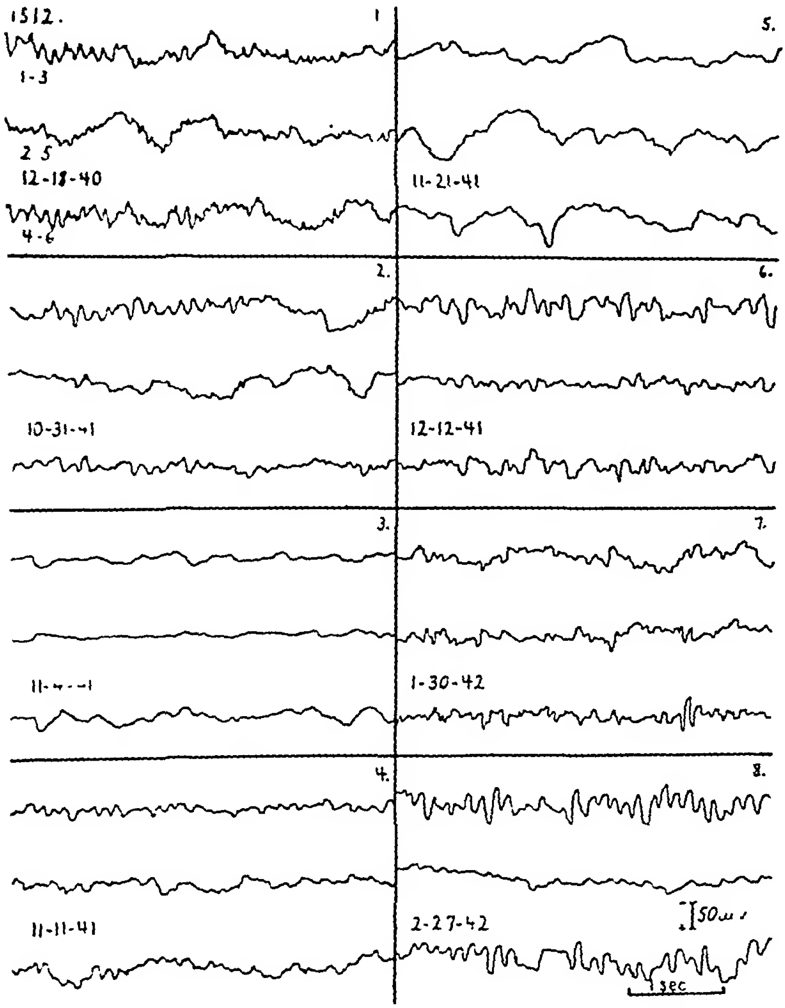

FIG. 2. Electroencephalograms from an immature *Macaca mulatta* (2.5–3.5 kg.) showing: 1, and 2, Normal pattern; 3, Effect of ablation of left area 6 on Nov. 3, 1941; 4, Recovery; 5, Effect of ablation of right area 6 on Nov. 18, 1941; Followed by return to preoperative pattern (6, 7, 8).

above the active tissue were slightly greater in amplitude than those above the ablation. It is to be remembered that the diameter of these monkeys' heads is 4–6 cm. and that the individual leads are never more than 2 cm. apart, a factor which makes localization less possible than on the human head.

The fine, rapid components of the EEG and the slower 8–10 sec. waves were unchanged after the first week. There was also as much variability in the pattern as had been present before operation. The effects of light, sleep, sound, etc., were as easily visible.

record was taken there was a change in the character of the potentials. This persisted and became intensified after removal of the second caudate during the sixth month. For the succeeding two years records taken at intervals have shown the picture now known to be characteristic of disturbances which follow lesions of the caudate and putamen.

In all records from animals with such ablations these characteristic changes have appeared although the degree of abnormality varies, possibly with size of the lesion: (i) A marked diminution in the amount of fast 15–20

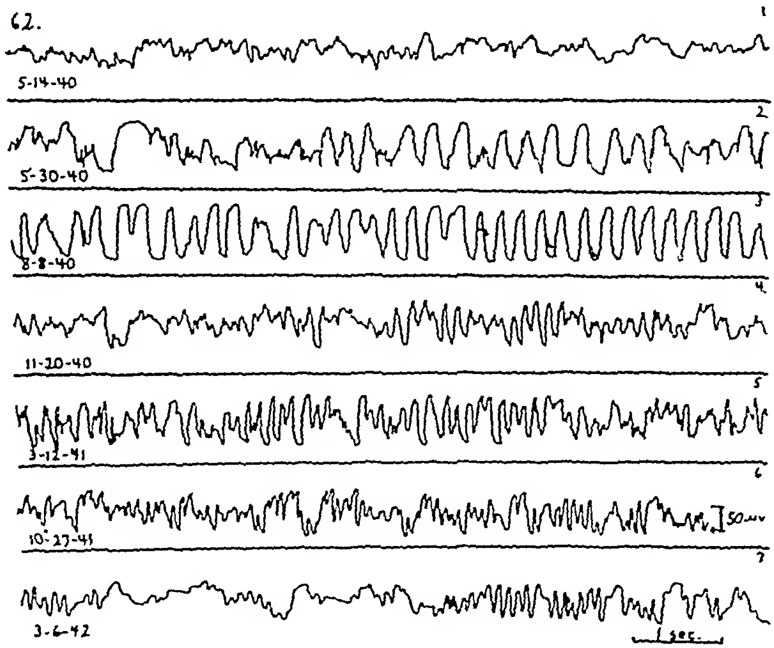

FIG. 4. Effects on EEG of ablation of head of left caudate nucleus on May 20, 1940 and of head of right caudate nucleus on August 16, 1940. This infant was born April 27, 1940. Hypersynchrony is invariably present. Note increase in rate characteristic of a normally developing animal during first two years of life.

per sec. rhythm which at times disappears altogether. (ii) An increase in the regularity of the pattern and a diminution in total variability. (iii) The most noticeable change is in the medium 5–10 per sec. rhythm which becomes accentuated and forms the "saw-toothing" described by Case and Bucy (1) or "hypersynchrony" as designated by Jasper (11, 13), (Fig. 3, 4, 5).

Such hypersynchrony may appear only in short bursts between periods of more or less normal record, or may continue for many minutes. It is sometimes entirely absent, and then may appear the next day in the record of the same animal. When present, it usually is seen in all leads, but at such times the amplitude is greatest in the frontal regions (1E, 4E) and in the interhemisphere records (1–3 and 4–6). In all leads, amplitude is increased during these periods of hypersynchrony. Although such changes have per-

the brain develops and grows, cortico-subcortical relationships are altered as they are in older animals with cortico-subcortical lesions.

C. Effects of lesions of caudate and putamen (4 monkeys). In contrast to the minimal effects of pure cortical lesions, ablation of head of caudate nucleus or of caudate and putamen produced very marked changes (Fig. 3). These changes were observed immediately after both unilateral and bilateral extirpations in 2 infants and 2 adults. Those produced in the infants were much greater than those in the adults, but of the same type. Figure 4 shows

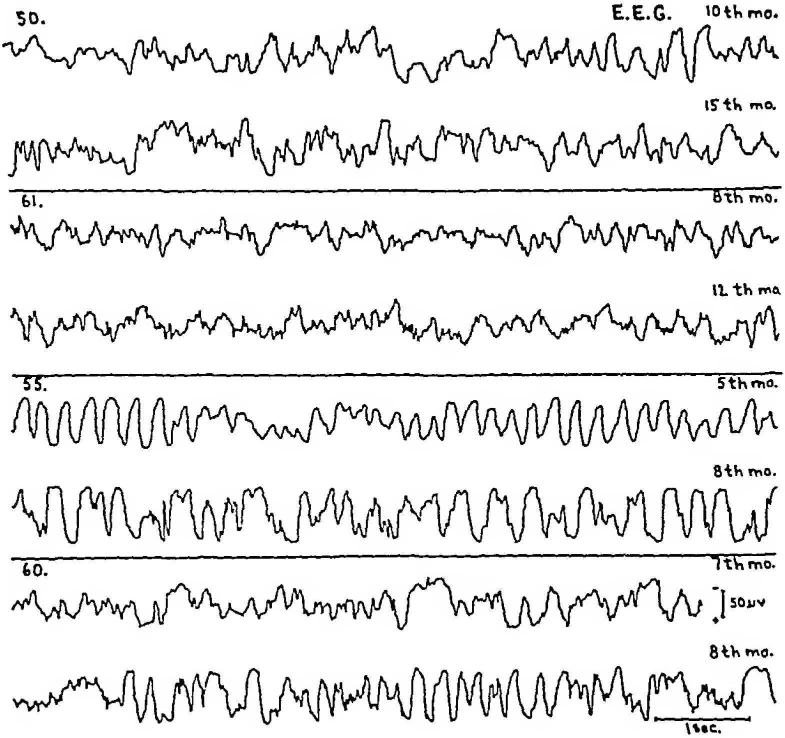

FIG. 3. Postoperative records from 4 young *Macaca mulatta*, the upper two (50 and 61) show nothing abnormal in EEG following cortical ablations (areas 4 and 6, bilaterally); the lower two (55 and 60) show marked hypersynchrony following bilateral ablations of areas 6 and caudate nucleus.

samples of the records from one such case. This macaque was born in the colony. EEGs taken on the fourth day and during the fourth week of life showed normal rate and amplitude for an infant at those ages. Fast and medium waves were rather variable as is normal at this time and there were occasional slow waves at about 2–3 per sec. superimposed on the 4–5 per sec. faster rate.

During the fourth week of life the left caudate nucleus was removed by suction via a small hole in area 8. Two days after operation when the next

Hypersynchrony appeared as the most pronounced change. It was extremely marked, being present at times only in short bursts, but running usually throughout the records and being present in all leads. The amplitude of the leads to ear (1E, 2E, etc.) was always very great, that of the potentials

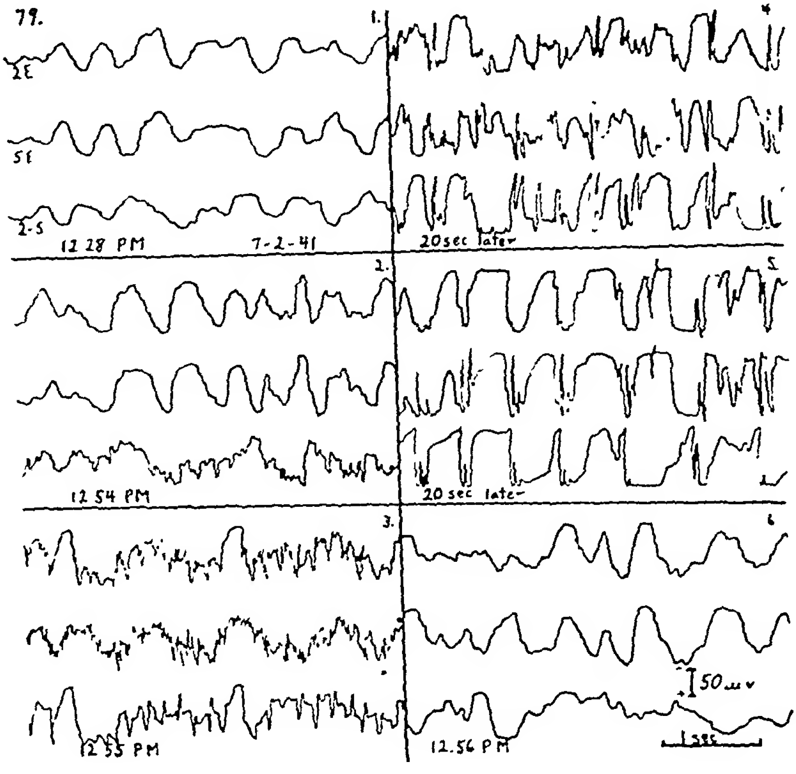

FIG. 6. Episodes from an attack, of tonic clonic generalized epilepsy in a *Macaca mulatta* 48 hours after bilateral ablation of area 6 and caudate nucleus.

between the hemispheres (1–4, 2–5, 3–6) decreased. The hypersynchrony, then, did not appear definite in the interhemisphere leads of low potential, while it was extreme in the intra-hemisphere recordings. The fast potentials during these episodes of hypersynchrony are invisible, and at all other times are diminished. There are no slow potentials, so that the appearance of the record is characteristic.

Records of epileptic attacks have been made in five of these animals, three of them infants, one a young animal and one a nearly mature female. Four of these five had clinical grand mal attacks at the time when the records were being taken. The fifth, the older animal, has never been observed in an epileptic attack, although its other clinical symptoms and the nature of its lesion are the same as those of the others. In each animal, attacks appeared on the day after operation and have continued for some days or

sisted for as long as two years, the longest period for which animals have been kept following such operations, they tend to be more marked in the weeks immediately after operation and become less extreme with time. These animals with lesions restricted purely to the basal ganglia have no clinical signs of neurological deficit of any kind.

D. Effect of combined lesions of cortex and basal ganglia (15 monkeys). These combined lesions produced the most definite and extreme changes in EEG which were noted. The cortical lesions were extirpations of either areas 4 or 6 or both. They were made in conjunction with ablations from

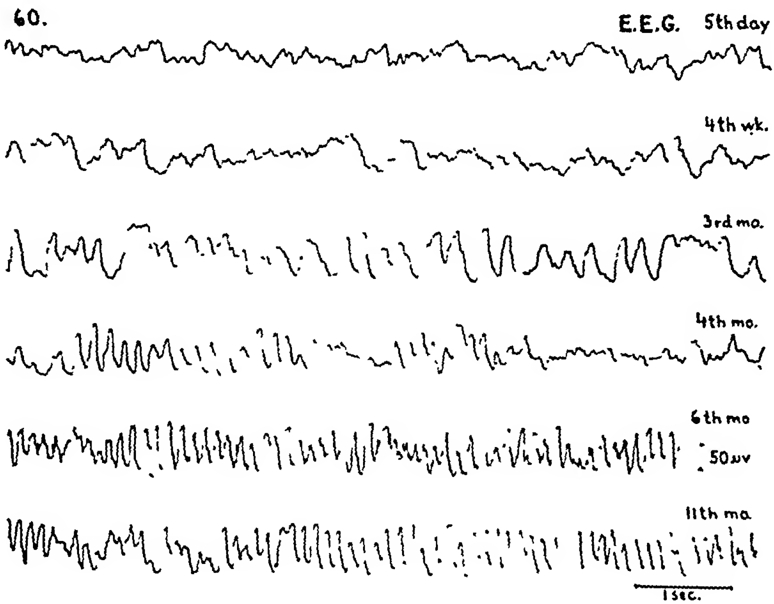

FIG. 5. Hypersynchrony appearing in an infant *Macaca mulatta* which had seriatum ablation of areas 4 and 6 and caudate nuclei; from left side during 5th week and from right during 5th month of life. Note increase of rate with age.

caudate or putamen or both. In every instance the change appeared immediately after operation, (many of the records were taken 24 or 48 hours after the lesion had been made) and persisted for as long as the animals lived (2 years in some cases). They were often more extreme immediately following operation and became less pronounced with time. They were always more pronounced in those animals operated in infancy (6 monkeys) than in those operated at a later date (9 monkeys). Epilepsy when present, occurred only during the first weeks following operation and later disappeared.

Figures 5, 6 and 7 show the type of alterations in EEG which are characteristic of these combined lesions. They are, in general, similar to those described by Jasper in his appraisal of the cortical potentials of epileptic humans (11).

throughout a period of more than three weeks, had records indicating various types of increased excitability of cortex. In the illustration excerpts have been chosen showing isolated spiking of one lead only, spiking of all leads, and parts of a generalized attack. This animal is now four months postoperative, and continues to show hypersynchrony, but without epilepsy. In this monkey (Fig. 7) the focus of excitability could be at times isolated to one area of cortex. At other times it spread to involve all other areas as well. Thus, by changing combinations of the leads, it could be seen that the area of largest and most constant spiking was in the region of lead 2, or just above the ablation on the left side.

Discussion

A comparison of the pre- and postoperative EEGs of 41 monkeys with lesions in cortex and basal ganglia has brought out some consistent and at times surprising points of interest. The most striking of these is that there is little or no change in the pattern of cortical potentials if the cerebral cortex alone is damaged, while lesions of the basal ganglia together with cortex, produce profound and permanent rearrangement of this pattern.

Although it is commonly thought that the electroencephalogram is a picture of the sum of *cortical* potentials alone, it has been shown not only by the present series of experiments but by much previous work, that activity of cortex and basal ganglia are intimately related. It is an old and consistent finding that stimulation of the cerebral cortex and of the cortex alone can produce epilepsy, and that no stimulation of basal ganglia alone results in any type of motor response (22). Yet combined stimulation (19) or ablation (15, 16) of basal ganglia and motor areas of cortex will materially alter the pattern of the motor response which is integrated through the cortex. Furthermore, it is extremely difficult to produce experimentally any type of permanent or recurrent epileptic state by injury to cerebral cortex alone although, recently this has been successfully done as reported by Pacella, Barrera and Kopeloff (20). To this can be added the well known clinical finding that epilepsy develops frequently only long after the cortical injury has been incurred. Foerster and Penfield (10) have shown that such epilepsy which first appeared in a large series of cases of brain injury many years after the initial trauma is due to scar formation and retraction distorting the ventricles as shown by pneumoencephalogram. Such distortion would very probably alter conditions in the basal ganglia since the caudate nuclei form the lateral boundaries of the lateral ventricles. That excision of cortical scars (21) abolishes epilepsy in these cases must be explained by the fact that the excitable foci for the attack lie in cortex, and that no epilepsy is possible without active cortical cells lying somewhere in the reverberating circuit of electrical activity.

That the cortex and in particular the motor areas of the frontal lobe, areas 4 and 6 of Brodman and the strip area 4-s (9) lying between them, are intimately concerned in integration of "voluntary" motor acts has been shown in various ways. Combined ablation of these areas with caudate pro-

weeks. In each, epilepsy was present at times, but absent at others. Thus, daily records would show, on two or three successive days, either grand mal or the "spiking" records which indicate cerebral hyper-excitability. There might then be a day or several days without epileptiform potentials and then a seizure would be found at a later date. In each animal the epilepsy disappeared by the end of the first postoperative month but the hypersynchrony

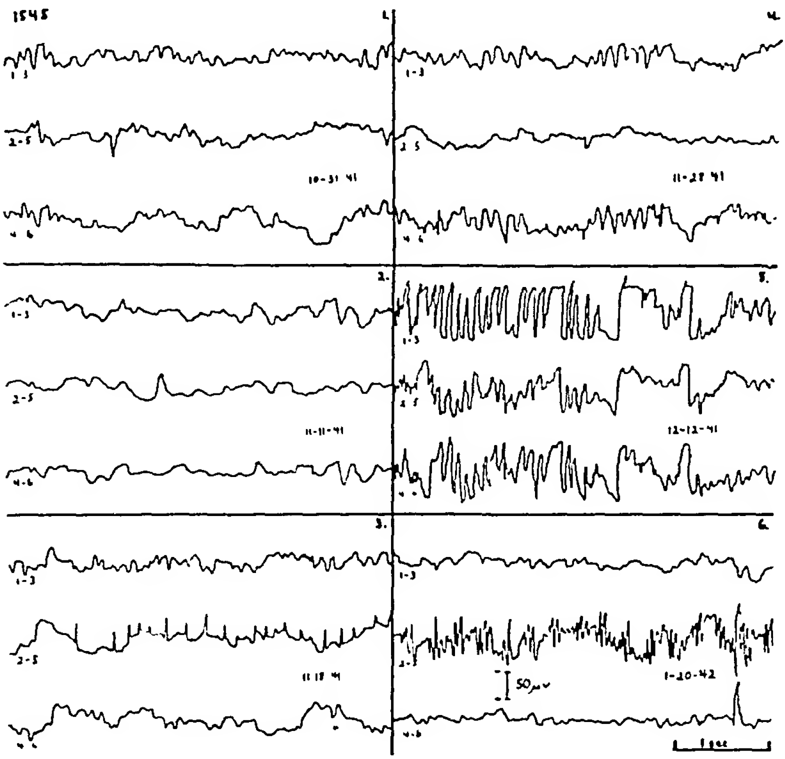

FIG. 7. Changes in EEG following bilateral ablation of area 6 and caudate nucleus on Nov. 10, 1941 in a *Macaca mulatta* (weight—4.0 kg.) 1, Normal; 2, Postoperative slowing; 3, Focal irritation in leads 2–5; 4, Hypersynchrony; 5, Epileptic attack; 6, Focal irritation persisting 6 weeks later.

remained. All these animals had, in addition to epilepsy, marked motor deficit; tremor, spasticity and impairment of skilled voluntary movement.

Figure 6 gives excerpts from a record taken throughout an attack of generalized epilepsy. During this period the animal showed first clonic and then tonic generalized convulsions. The entire attack lasted about six minutes. It was one of many witnessed during the first three days subsequent to simultaneous bilateral removal of areas 6 and head of caudate nucleus from a 4-week-old infant.

Figure 7 shows the records of several days taken after a similar ablation. This is the animal which was never seen to have clinical epilepsy, yet

The most important finding brought out by these investigations has been the production of epilepsy both clinically and in the EEG and its relation to the basal ganglia. All the changes in pattern, hypersynchrony and irregularities, as well as true epileptic seizures can, according to the descriptions given by Jasper (11, 13) and Gibbs and Gibbs (7), be attributed to the epileptic state either between attacks or during an acute episode.

The conditions essential to cause true epilepsy in these animals are not clear, for in only five out of fifteen instances in which bilateral ablation of area 6 and the caudate nucleus has been performed did these attacks appear. However, in every case the affected animal has been one which has showed the most severe clinical symptoms of tremor and spasticity. It is likely therefore that size of lesion may determine this epileptic factor. In contrast those monkeys with pure caudate and putamen lesions, which had minimal clinical symptoms all developed hypersynchrony but no epilepsy.

The fact that monkeys operated upon in infancy have uniformly exhibited more striking changes than have those operated upon at a later age is consistent with what is known of similar conditions in man, particularly if these changes in the monkey are related to epilepsy. For it is well known that infants are particularly susceptible to epilepsy under almost any condition that can bring on seizures whether fever, non-specific trauma or focal cerebral injury. In all infants there must then be some condition of the cerebral cortex or basal ganglia which permits synchronization of cortical potentials into patterns characteristic of epilepsy more readily than in the adult. The greater tendency of the young of man to other disorders of the basal ganglia such as chorea and athetosis is also known as is the greater capacity for reorganization of the cerebral cortex for physiological activity (14).

Conclusions

Lesions have been made in cerebral cortex and basal ganglia of 41 monkeys, and electroencephalograms (EEG) recorded before and after operations.

1. In every instance there is a temporary change in EEG which appears during the first or second postoperative day, and which consists of flattening and slowing of the waves of medium frequency. It is transient; it is independent of the specific area injured; but it is more pronounced following larger lesions.

2. In acute experiments under dial anesthesia, lesions of cortex or basal ganglia produce no change in EEG.

3. Following lesions restricted to cerebral cortex there is no significant change in EEG except the transient one described above.

4. There was no focal effect of lesions of the cortex, and usually none following combined lesions of cortex and basal ganglia. In a few instances focal epilepsy could be seen.

5. Lesions of head of caudate nucleus or putamen, or of both, were followed by marked changes in the pattern of cortical potentials. Hypersyn-

duces in the monkey or chimpanzee a type of motor disorder which is characteristic for this combined ablation (16). To this should be added the information obtained by Dusser de Barenne and McCulloch (3, 4) that there are reverberating circuits defined by strychninization of various areas of cortex which pass from cortex directly to caudate and putamen and thence back to cortex either directly or via thalamus. Interruption of these pathways either by excision of area 6 and caudate nucleus or by large bilateral excision of caudate and putamen alone will be followed, in the monkey, by the appearance of dyscoordinations and dysrhythmias which are another indication of disturbance in rhythmic pattern.

There is some clinical evidence that the basal ganglia affect the EEG, for Gibbs and Gibbs (7) have seen changes in records from patients with athetosis and chorea although those with Parkinson's syndrome did not show similar abnormalities. Dynes and Finley (2) studying narcolepsy report characteristic patterns which are altered by the benzedrine drugs.

In the light of previous experience with man, it is surprising that there are no focal signs in the EEG of the monkey following specific lesions. Moreover, when changes develop following combined lesions of cortex and basal ganglia they were, with few exceptions, manifest in all leads. Only when true epilepsy was present or in the preepileptic state of the same animals did occasional isolated foci of unusual excitability occur.

The non-specific reaction of the potentials to trauma at any point of the central nervous system thus far injured may be compared to the changes found in man after injury to the head (12, 18). There is the same slowing of rate and smoothing out of amplitude and pattern. The degree and rate of recovery is related in the same way to the extent of the lesion. That concussion is not the same as experimental ablation probably accounts for the fact that the picture in man is complicated in some cases by the appearance of epileptiform patterns (12) although this does not appear in the experimental monkey.

There are data from several aspects of this investigation which show that *after* and not *during* the time of cortical excision, changes take place which alter cortical potentials. First, no alteration in pattern was produced in acute preparations immediately following excisions of any portion of the nervous system although removal of some of these same areas was followed by profound changes in the EEG of the chronic preparations. Two factors might effect this difference. Either there must be some rearrangement of remaining tissue which accounts for the eventual changes, or else, the anesthesia (dial in these cases), may alter the acute picture in some way so that immediate changes are not apparent. The matter could be easily further investigated, and must be before any definite conclusion can be reached.

Other signs of rearrangement after operation appear in relation to the non-specific changes which follow cerebral operation. As described above, these changes are sometimes not apparent on the first day after operation but only on the second day. In this instance they may be correlated with the reaction of tissue to injury.

MODIFICATION OF CORTICAL ACTIVITY BY MEANS OF INTERMITTENT PHOTIC STIMULATION IN THE MONKEY

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IT HAS BEEN SHOWN by Bartley and by Bishop that the cortical activity (electrical) of the rabbit may be markedly altered in amplitude and rate by intermittent photic stimulation of the retina. Cortical responses approach their maximal amplitude when the flashes of light occur at the frequency of the alpha rhythm (2, 6, 7). However, the alpha frequency may itself be raised or lowered in the rabbit under certain conditions by corresponding rates of stimulation (5). In the cat, Bishop and O'Leary have found that while the cortical activity may be enhanced in amplitude by electrical stimulation of the optic nerve at the alpha frequency, the results for photic stimulation were less distinct (9, 8). In man, Adrian and Matthews (1) and others have found, as in the case of rabbit, that the alpha rhythm may be substantially raised or lowered in rate by corresponding rates of photic stimulation. The present investigation affords evidence that in the monkey the cortical activity may be markedly altered in rate and amplitude by intermittent light, and that with the same luminous flux, maximal cortical responses are obtained at particular frequencies of stimulation.

METHOD

Experiments were carried out on 12 adult monkeys (*Macaca mulatta*). Electroencephalograms were simultaneously recorded on paper tape from both occipital regions by means of the three-channel amplifying equipment† and procedures (adapted for monkey) described by Case and Bucy (11). Flash intervals were also recorded continuously on the tape by means of a photocell pickup working into the third amplifying channel.

Stimulation was carried out with the monkey in a darkened, electrically shielded cage. The animal was attached to an animal board and placed before an aperture in one side of the shielded cage in such a manner that flashes of light introduced through the aperture gave an equal illumination to both eyes of 120 footcandles. Light from a tungsten source was interrupted at various frequencies by means of an interposed episcotister driven by a DC motor. Light-dark ratio was 1/1.

Quantitative analysis was made of all records. At intervals of one foot of tape, each record was sampled for 10 successive waves until 25 such groups were obtained for each animal (tape speed of 3 cm. per sec.). Each set of 250 waves was then measured for amplitude in units of 0.25 mm. under a reading lens giving 2.5 times magnification. The measurements were then separated into two groups depending upon whether or not the cortical frequency corresponded with the flash frequency. For convenience of comparision, the total amplitude per second was calculated for each frequency by multiplying the average amplitude by the frequency. The values for cortical frequencies which did and did not correspond to flash frequency are the basis for the curves shown in Fig. 1, 2 and 3. Ac-

* The animals employed in this investigation were purchased by funds granted to one of the authors (Walker) by the Ella Sachs Plotz Foundation.

† The authors are indebted to Dr. Theodore J. Case for the use of this equipment.

chrony of the 8–10 per sec. waves became intensified, and the 15–20 per sec. waves became less marked or vanished.

6. Combined lesions of the motor areas of the cortex with the basal ganglia caused the most marked changes. Hypersynchrony appeared and persisted for as long as two years. True epilepsy, both clinical and in EEG were found in 5 out of 15 animals.

7. All the lesions which caused changes in EEG produced the most extreme changes in those animals which had been operated upon in infancy.

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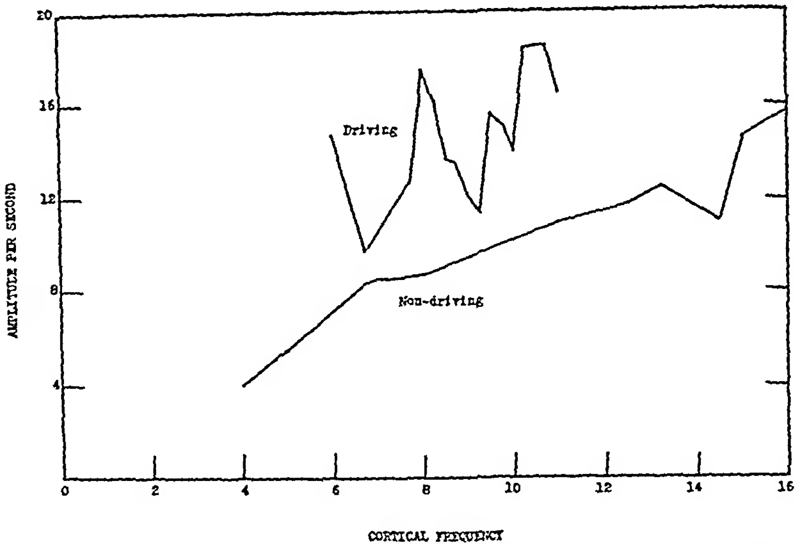

FIG. 1. Graph for monkey X showing the amplitude per second of cortical activity during intermittent photic stimulation (upper curve) when cortical frequency corresponds with flash frequency and (lower curve) when it does not correspond with flash frequency. The amplitude per second was calculated by multiplying the average amplitude of 10 successive cortical waves by their frequency (cf. text).

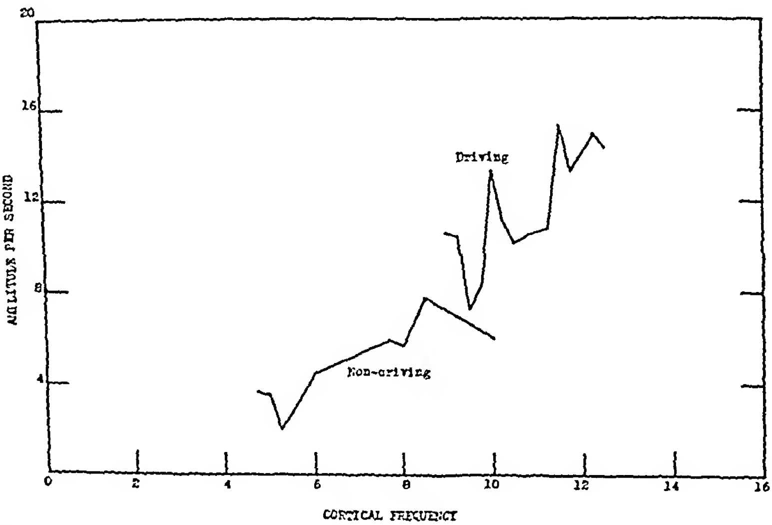

FIG. 2. Graph for monkey Y similar to Fig. 1 but showing relatively discrete driving and non-driving spectra of cortical frequencies.

above for the tungsten source of light. In comparison then with the tungsten source, with which driving was produced in all twelve monkeys, fluorescent light was effective in only 43 per cent of a second group of 21 monkeys. The possible role of such factors as flash intensity, light-dark ratio and spectral composition in producing this difference is considered in a separate publication.

curacy of amplitude measurements was checked by re-measuring 200 waves from a tape selected at random and was found to be better than 96 per cent in terms of the average deviations. Both ascending and descending series of flash frequencies were presented in each experiment to reduce the probability of chance correspondence between cortical frequency and flash frequency.

Results

Driving effect of intermittent light. Substantial evidence of an effect of intermittent photic stimulation upon the cortical activity was found in the record of every monkey. This effect was reflected over a range of several cycles in two ways: (i) by increased amplitude per unit of time of the cortical activity, and (ii) by regularization or increased stability of the cortical rhythm. Over the range in which driving was produced, the effect was present on an average of 55 per cent of the time for the group as a whole and ranged from 28 to 78 per cent for individual monkeys. Representative curves indicating the magnitude of the driving effect are shown in Fig. 1, 2, and 3.

Range of driving effect. The range of frequencies at which driving occurred varied in different animals from 7.5 cycles to 2.0 cycles per sec. and averaged 5.0 cycles per sec. for the group as a whole.

Relation of amplitude per unit of time to flash frequency. Examination of the amplitude maxima obtained during driving (*i.e.*, with cortical frequency synchronous with flash frequency) revealed that they tended to cluster in a limited range. A plot of the maxima against frequency is shown in Fig. 4. It may be noted that for the twelve monkeys (plus a repeated record made on one animal) 9 amplitude maxima fall within a frequency range of one cycle per sec., *i.e.*, within the range from 10.5 to 11.5 flashes per sec. This fact becomes of particular interest when contrasted with the non-driving condition. Here the amplitude maxima were found to be distributed over a much wider range (7 to 29 cycles per sec.) with no suggestion of clustering. In no instance did more than two animals have amplitude maxima falling within one cycle of the same frequency.

Regularization of rhythm. Regularization or stabilization of rhythm was a marked feature of the records obtained under conditions of driving as contrasted with non-driving. This is illustrated in the sample records reproduced in Fig. 5.

Production of multiple frequencies. Further evidence of the effect of the intermittent flashes was the not uncommon appearance of cortical frequencies exactly double or triple that of a given flash frequency. A similar result has been obtained by other investigators (cf. 1, 2, 8).

Auditory component ineffective. Control records obtained with the apparatus in operation but without the light flashes revealed no evidence of driving.

Effectiveness of fluorescent light in driving cortical activity. Preliminary investigations were made of the effectiveness of a G.E. 15 watt daylight fluorescent lamp, made intermittent at various frequencies by means of a multivibrator circuit, upon driving of cortical activity. Substantial driving was obtained in nine monkeys with flashes of 44 footcandles in brightness with a light-dark ratio 1/1. In twelve monkeys, no driving was produced by fluorescent light under the conditions otherwise identical with those described

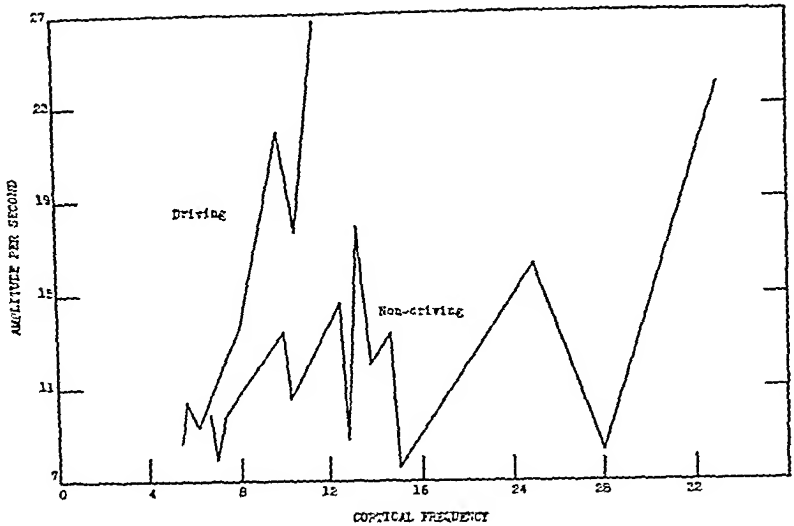

FIG. 3. Graph for monkey W similar to Fig. 1 but showing a narrow driving spectrum and a broad non-driving spectrum of cortical frequencies.

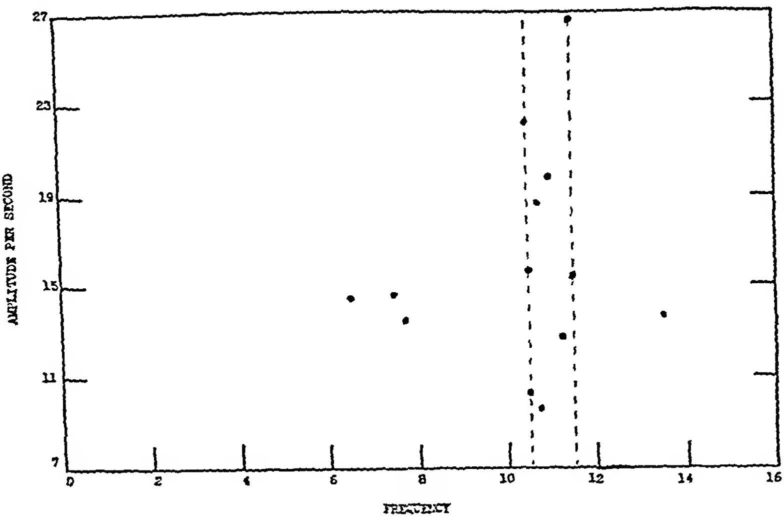

FIG. 4. Graph showing the clustering of the amplitude maxima, obtained under conditions of driving in twelve monkeys (with repeat record on one animal), at a frequency of approximately 11 per sec. No clustering was found under conditions of non-driving (cf. text).

driving occurs at the alpha frequency as indicating the operation of a common mechanism in the brain rather than at the periphery (5). If this proves to be sound, then it seems probable from the results obtained in our experiments that the region of maximal enhancement of brightness of intermittent light for monkey will be found in the neighborhood of 11 flashes per sec., since maximal alteration of the cortical activity occurs in this region.

Discussion

Little is known concerning spontaneous frequencies which occur in the electroencephalogram of the monkey. For *Macaca mulatta* frequencies of 7 to 8 per sec. have been reported by Kennard and Nims (13) as characteristic of older animals, in contrast with frequencies of 4 to 5 per sec. seen in the records of animals 6 to 8 months in age. Greater variations were found in the records of the older animals. It is of interest that amplitude maxima under driving conditions were found by us to cluster between 10.5 and 11.5 cycles per sec. Only three of our animals produced amplitude maxima in the region of 6 to 8 per sec. and in one animal maximum amplitude was reached at 13.7 cycles per sec. (cf. Fig. 4). Under non-driving conditions it was found that waves of large amplitude occur at frequencies as high as 29 per sec. (cf. Fig. 3). No driving was found above 13.7 per sec. and only two monkeys drove above 12 per sec. There was in fact a marked tendency noted in the records for the upper limit of driving and the amplitude maxima to fall at the same frequency. No explanation for this abrupt cutoff in driving can be offered at this time.

The sensory resultant for man of frequencies of intermittent light below fusion is flicker. Two regions in the range of flicker are known to produce *enhancement* of apparent brightness. The first of these was reported by Brücke (10) in 1864 to be in the region of 17 to 18 cycles per sec. He observed that the white areas of a rotating disc made up of black and white sectors became more brilliant at this rate of rotation than when stationary. The second range of frequencies known to produce brightness enhancement was reported by Bartley (3) in 1938. Using transmitted light, Bartley measured the average brightness level for successively lower flash rates. He found that the brightness of the "average" level begins to rise until it reaches, then exceeds a level equivalent to that obtained with continuous illumination. Maximum enhancement is reached at 8 to 10 flashes per sec. At this point the apparent brightness is the reciprocal of the fused brightness of the intermittent field. Bartley's findings on brightness enhancement were recently confirmed by Halstead (12) who eliminated the possible origin of the effect in the pupillary and accommodative reflexes by studying these relations in a normal subject with these reflexes eliminated by scopolamine.

It seems probable that the Brücke effect and the Bartley effect are distinct phenomena in the range of flicker. The Brücke effect is obtained by judging the bright phase of the fluctuating component of the intermittent source. The Bartley effect is obtained by judging the steady component of an intermittent test-field but does not appear when the fluctuating field has an illuminated surround (3, 4). The Brücke effect was obtained under conditions in which the fluctuating field had an illuminated surround.

It is not known whether either or both of these effects are operative as mechanisms in the brightness vision of the monkey. Bartley has interpreted the correspondence between the region of maximal brightness enhancement and the alpha frequency of 8 to 10 per sec. in man and the fact that optimal

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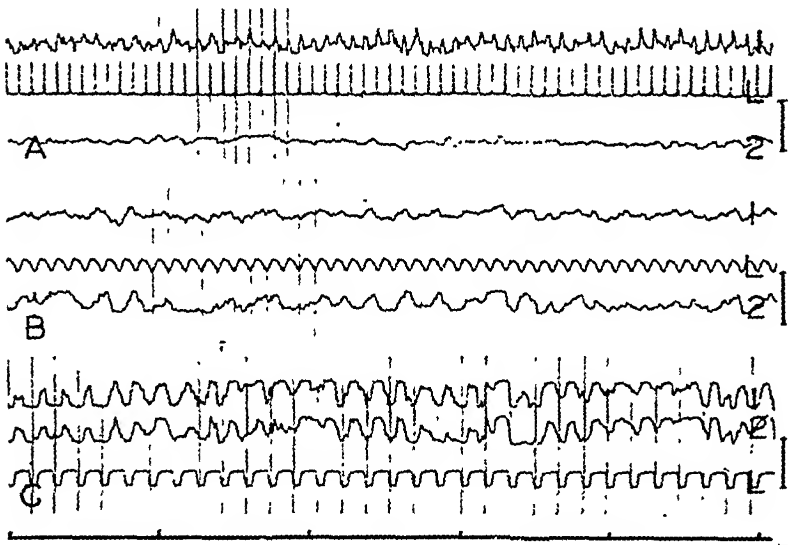

FIG. 5. Representative electroencephalographic records showing:
A. Driving at a frequency of approximately 11.5 per sec. in the left occipital lead only.
B. Non-driving bilaterally at a flash frequency of 9 per sec.
C. Driving in both occipital leads at a frequency of 6 per sec.
(In each record 1—left occipital lead, 2—right occipital lead, L—light flashes as recorded from a photoelectric cell. The time in seconds is indicated at the bottom of the figure. The units at the right of the tracings indicate a deflection of 300 μV.)

SUMMARY

Evidence was found of an effect of intermittent photic stimulation upon the cortical activity of the monkey (*Macaca mulatta*).

The effect was reflected by increased amplitude of cortical response and by stabilization of cortical rhythm. The effect is similar to the driving effect reported by Bishop and his co-workers for rabbit and cat and by various investigators for man.

The range of frequencies found to produce maximal cortical responses in monkey was from 10.5 to 11.5 per sec.

The driving effect was found to be present in 100 per cent of twelve monkeys.

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RELATION OF FIBRILLATION TO ACETYLCHOLINE AND POTASSIUM SENSITIVITY IN DENERVATED SKELETAL MUSCLE

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WHEN the motor nerve to a skeletal muscle is cut the muscle involved undergoes several distinctive changes. At about the time the nerve degeneration is complete fibrillation commences. This is accompanied by characteristic action potentials (1, 3, 6, 7). Concurrently the muscle also develops an increased sensitivity to acetylcholine. Close arterial injection of a large dose of this substance (1) into a normal muscle produces a brisk contraction. Distant injections are without effect. With denervated muscle, however, administration of a minute dose of acetylcholine, by either the intra-arterial or intravenous route, produces a characteristic response (1). This consists of an initial "quick" movement of the muscle, accompanied by an outburst of action potentials, and is followed by a prolonged contracture. A similar difference in the nature of the contraction in normal and denervated muscles was observed following the arterial injection of potassium chloride but no difference in sensitivity was reported (2).

Quinine depresses, but does not completely abolish, the response to acetylcholine administration in cats (4). Oester and Maaske (5), working with dogs, found that large doses of quinidine almost completely abolished the effect of intra-arterially injected acetylcholine. In neither of these investigations, however, was this acetylcholine antagonism considered in relation to the effect of quinidine on fibrillation. In previous investigations (6, 7) fibrillation was abolished in the denervated muscle of the rat with adequate dosage of quinidine. The experiments to be reported here were undertaken with the object of comparing this property of quinidine with its action on the sensitivity of these muscles to acetylcholine and potassium in the hope of effecting a further elucidation of the genesis of fibrillation in denervated skeletal muscle.

METHODS

Albino rats, weighing 200 to 350 gm., and on an adequate diet, were used throughout. The gastrocnemius-soleus muscle group on one side was denervated, under ether anaesthesia, by removing a length (0.5 cm.) of the sciatic nerve. The animals were tested between the 6th and 14th days after denervation.

Under light ether anaesthesia, a skin flap was cut, and the tendon of the denervated muscle group dissected out and detached from its bony insertion. The lower limb on that side was then immobilized with metal clamps running from rigid uprights to the femur and foot. A cord from a small hook through the muscle tendon was attached to an isotonic lever writing on a smoked drum. The action potentials were led from the muscle belly through

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tentials of fibrillation (Fig. 1, a) were readily detected by ear and photographed (6). Intra-arterial injections of acetylcholine produced results similar to those of Brown (1). There was first a rapid contraction accompanied by an electrical outburst ("quick phase"). This was followed by a prolonged slower mechanical movement during which all action potentials, including

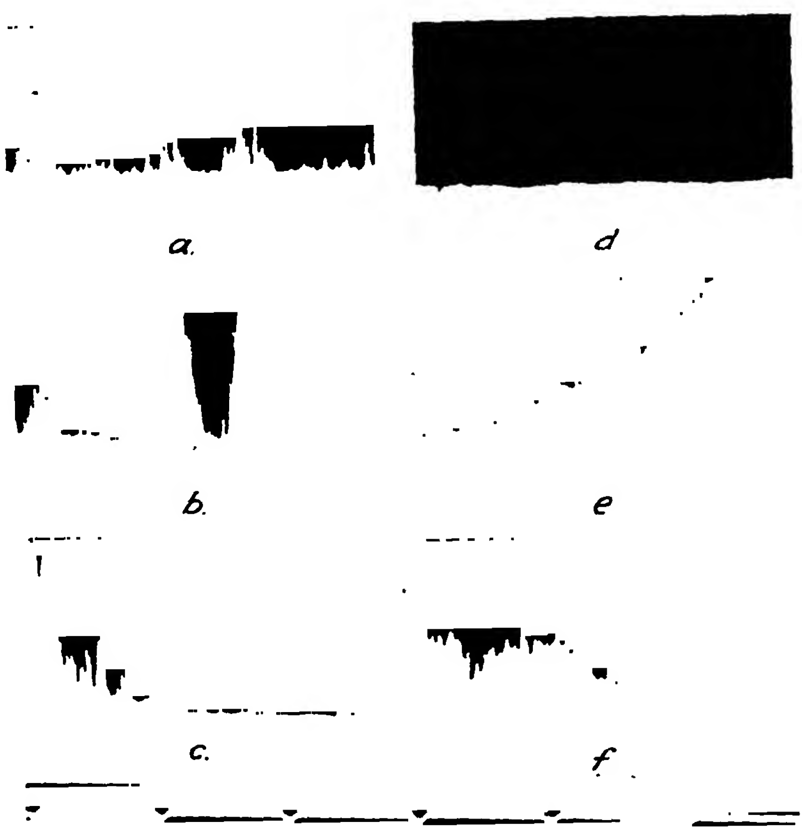

FIG. 2. Action potentials from gastrocnemius-soleus muscle of rat 8 days after denervation. (a) Fibrillation; (b) intra-arterial injection of 2.0γ acetylcholine; (c) 2 sec. after (b). (d), (e), (f) Similar records after 6 mg. quinidine sulphate given intra-arterially.
Time; 1 division = 0.2 sec.

those of spontaneous fibrillation, were abolished ("contracture phase"), or were too slow to be recorded by our apparatus. The duration of this second phase varied with the acetylcholine dosage and, with the smallest doses (0.02–0.002γ), was transient or absent (Fig. 1, a, b, c, d). With larger doses (2.0γ–2 mg.), the electrical outburst with the large initial contraction blocked the amplifier temporarily (Fig. 2, a, b, c) before the prolonged second phase appeared. The first three tracings of Fig. 3 are representative of mechanical records from such experiments. The two-phase response with

two fine needles and amplified. They were recorded photographically and rendered audible through a loud speaker. With the abdomen open, the iliac artery supplying the opposite limb was clamped off. All injections were then made into the lower aorta in constant volumes (0.25 cc.) and at a fairly constant rate. The drugs were in aqueous solutions. Acetylcholine bromide was used in various dilutions giving a dosage range from 0.002 γ to 2 mg. Potassium chloride was used in solutions containing 0.2, 2.0, and 20 mg. per cc. The quinidine solution contained 25 mg. quinidine sulphate per cc. The mechanical and electrical responses of the muscle to acetylcholine or potassium were noted before and after quinidine administration.

Results

1. *Effect of quinidine on fibrillation and acetylcholine sensitivity.* This series was made up of 29 animals. In all of them the characteristic action po-

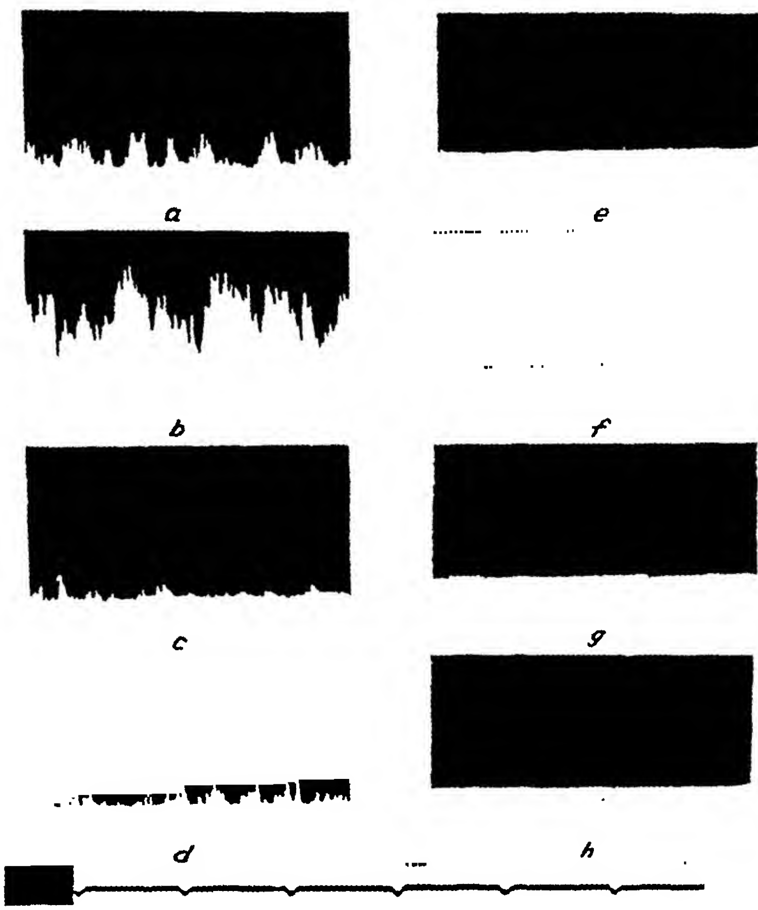

Fig. 1. Action potentials from gastrocnemius-soleus muscle of rat 8 days after denervation.

(a) Fibrillation; (b) intra-arterial injection of 0.02γ acetylcholine; (c), (d), 2 and 5 sec. after (b).

(e), (f), (g), (h) Similar records after 6 mg. quinidine sulphate given intra-arterially.

Time; 1 division = 0.2 sec.

effect. The mechanical counterpart is seen in Fig. 3, where, after quinidine, only partial reduction of contraction height occurred. With larger doses of acetylcholine (up to 2 mg.) there was only slight reduction in the outburst of action potentials, and no differences in the mechanical records were seen.

2. *Effect of quinidine on fibrillation and potassium sensitivity*. The relative effects of quinidine and potassium were studied in a similar fashion in 12 experiments. Here, the results were less clear-cut than was the case with acetylcholine though the general trend was the same. In the first place, though present, there was much less difference between the sensitivities of normal and denervated muscles to potassium administration. This is seen clearly in Table 1 where the minimal effective doses are compared for the

Table 1. Minimal effective dosage of potassium chloride producing mechanical movement in denervated and normal muscles.

	KCl dosage		
	20 mg.	2.0 mg.	0.2 mg.
Denervated muscle	1 experiment	4 experiments	7 experiments
Normal muscle	5	5	0

two types of preparation. Although one denervated muscle required 20 mg. of potassium chloride to elicit a response, yet in most cases 0.2 mg. produced a contraction. On the other hand, 5 normal muscles were effectively stimulated by 2.0 mg. of potassium chloride, 5 required 20 mg., and none showed a response to the smaller dosages.

After effective quinidine arrest of fibrillation, the sensitivity of denervated muscle to potassium administration was reduced in a fashion similar to that seen with acetylcholine. Doses of 2 mg. or less were ineffective. With higher concentrations of potassium chloride, however, considerable variation was seen. The response to injections of 20 mg. was unaffected by the quinidine in 3 cases. In 4 cases, some reduction was seen and in 5 instances the potassium was completely ineffective.

Discussion

In previous papers (6, 7) it has been shown that the fibrillation resulting from denervation of skeletal muscle can be abolished by adequate administration of quinidine. When this substance is injected intraperitoneally there is a diminution, in many cases a complete cessation, of fibrillation which lasts up to 9 hours. Denervated muscles are much more sensitive to acetylcholine than are normal muscles (1). On this basis it has been suggested (3) that fibrillation is the result of an asynchronous response to the acetylcholine normally present in tissue fluids.

The action potentials of fibrillation are of a degree comparable to those induced by the intra-arterial injection of relatively small doses of acetylcho-

2.0γ administration is evident. Such division is not seen, however, with the lower dosages. In this experiment, as in 21 out of the group, 0.002γ acetylcholine was the smallest effective dose. In the remaining 8 experiments, 0.02γ was necessary to evoke a response. In contrast, the smallest effective dose for normal muscle, was 2 mg.

Quinidine, in 6 mg. doses, produced an immediate cessation of fibrillation in all cases. The action potentials were abolished, with no evident mechani-

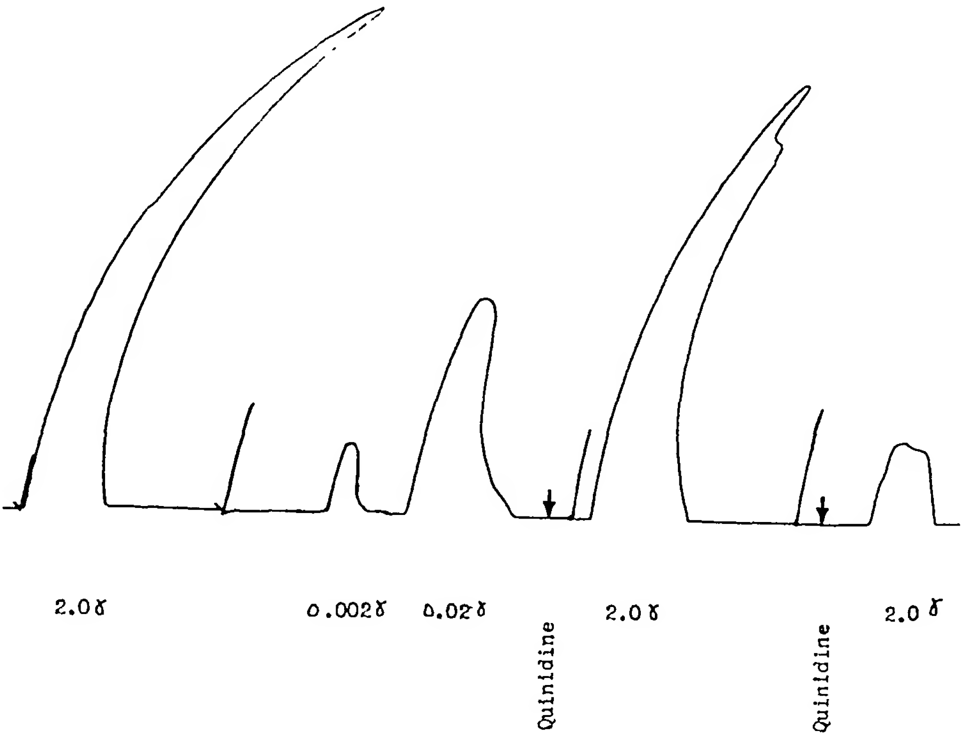

FIG. 3. Mechanical responses of denervated muscle to intra-arterial injections of 2.0, 0.002, 0.02, 2.0, 2.0γ (from left to right) of acetylcholine. At arrows, 6 mg. quinidine sulphate given intra-arterially 1 minute apart.

cal movement of the muscles. The duration of this arrest was not followed beyond a period of 15 minutes but it must, in most cases, have been considerably longer. During such periods of absent fibrillation, the effects of acetylcholine administration varied with the dosage employed. Small doses which normally produced definite contractions, were completely ineffective, mechanically and electrically. The flat baseline after quinidine (Fig. 1, e, f, g, h) was unaltered by the injection of 0.02γ acetylcholine. This dose, previously administered (Fig. 1, a, b, c, d), had produced a definite outburst of action potentials. The responses to higher concentrations of acetylcholine were opposed to a less degree. In Fig. 2 (d, e, f), although fibrillation had been abolished by quinidine, 2.0γ acetylcholine still produced considerable electrical

CEREBELLAR ACTION POTENTIALS IN RESPONSE TO STIMULATION OF PROPRIOCEPTORS AND EXTEROCEPTORS IN THE RAT*

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INTRODUCTION

THE AFFERENT TRACTS from the spinal cord to the cerebellum have been generally considered to carry proprioceptive sensory impulses exclusively. Every modern textbook of neuroanatomy assigns the spino-cerebellar tracts to the proprioceptive system. The tendency to link the cerebellum exclusively with proprioceptive sensation has been so marked that the term muscle-sense has been used by many interchangeably with spino-cerebellar when speaking of the impulses and connections from the spinal cord to the cerebellum. Although cerebellar deficiencies show themselves both clinically and in experimental animals as abnormalities of muscular tone and movement the anatomical connections in the spinal cord between the afferent neurons of the peripheral nerves and the dendrites of cells, which make up the spino-cerebellar tracts, are not necessarily restricted to the proprioceptive system. While no disturbance of touch, pain, hot or cold sensation has been observed following cerebellar lesions neither is the conscious appreciation of proprioceptive sensation interfered with when lesions are restricted to the cerebellum. Nevertheless it was with some surprise that one of us (2) observed that electrical stimulation of the saphenous nerve in the cat gave rise to cerebellar action potentials. Snider and Stowell (7) briefly reported that in the cat tactile stimulation of the forepaw and other parts of the body produced action potentials in the cerebellum. Our observations in the rat were apparently made at the same time as those of Snider and Stowell for the two reports appeared simultaneously (5).

Our purpose has been to compare the cerebellar action potentials produced by "physiological" stimulation of exteroceptors with "physiological" stimulation of proprioceptors. At the same time we wished to observe possible differences in the responses when different parts of the body were stimulated. Admittedly the rat is not the most suitable animal for the latter part of the study. Previous trials in the cat had been discouraging and when these potentials were readily obtained in the rat studies in this species were continued. After reading Snider and Stowell's report we have confirmed the fact that light touch of the paw and the nose of the cat will also produce action potentials in its cerebellum.

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line (Fig. 1, a, b, c, d) or of potassium chloride. They differ greatly from the electrical outbursts produced by large doses. Intra-arterial injections of quinidine, while having less effect on these large outbursts, completely abolish the responses to small doses of these substances (Fig. 1, e, f, g, h). Apparently quinidine, in concentrations which abolish fibrillation, renders denervated muscle unresponsive to doses of these other agents which ordinarily produce a comparable degree of excitation. These experiments, then, support the thesis that fibrillation arises from an increased sensitivity of the muscle to chemically-induced excitation and indicate that acetylcholine, potassium or both may be the cause of fibrillation. They do not, however, shed light on the possible relative rôles of acetylcholine and potassium in the production of the fibrillation of motor denervation.

Summary

Denervation renders skeletal muscle much more sensitive to acetylcholine and, less markedly, to potassium chloride. Small concentrations of these agents, when injected intra-arterially, produce action potentials comparable to and superimposed on those of fibrillation. This excitation is abolished by quinidine in doses which will arrest fibrillation. It is suggested, therefore, that the fibrillation seen in skeletal muscle after lower motor neurone denervation arises from an increased sensitivity of denervated muscle to chemically-induced excitation. The present experiments indicate that acetylcholine, potassium or both may be the usual causative agent or agents.

We are grateful for the interest taken in this work by Professor C. H. Best.

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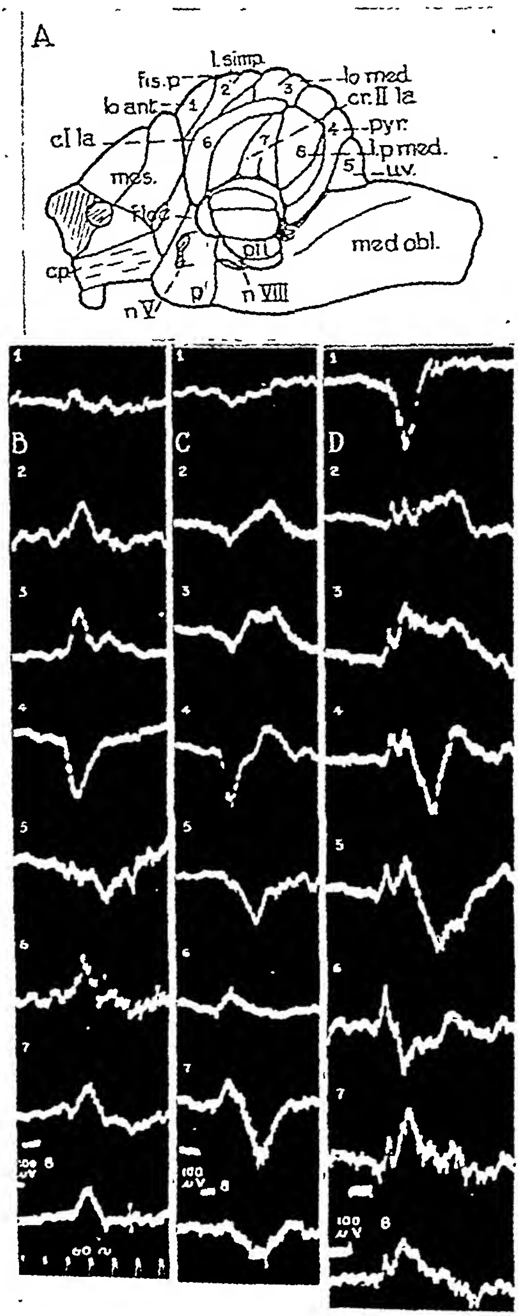

FIG. 1. A. Lateral view of the rat's cerebellum. Nos. 1 to 8 refer to the lead points for the corresponding records. c.p., cerebral peduncle; c.I. la., Crus I lobulus ansiformis; cr.II la., Crus II lobulus ansiformis; fis. p., fissura prima; floc., flocculus; lo. ant., lobus anterior (highest folium of culmen); lo. med., lobus medius (folium and tuber vermis); l. simp., lobulus simplex; l. p. med., lobulus paramedianus; med. obl., medulla oblongata; mes., mesencephalon (superior and inferior colliculi); n.V., trigeminal nerve; n.VIII, acoustic nerve; p. pons; pfl., paraflocculus; pyr., pyramis; uv., uvula. B. Potentials recorded following tapping the tendon of the homolateral quadriceps femoris muscle. A deflection downward in these and subsequent records indicated a positive potential. The instant of stimulation is not shown but all records in each column have been mounted so that the time sequence of one is comparable to another. Note an early positive potential only in the pyramis, B4. C. Potentials recorded following tapping the tendon of the homolateral triceps brachii muscle in the same animal. Note the largest early surface positive potential is led from the pyramis, C4. Other surface positive potentials of comparable amplitude are led from the uvula, C5, and Cruss II, C7. They occur respectively 17 and 21 msec. later than the one led from the pyramis. D. Potentials recorded following moving the hair on the back of the same animal. Note the early surface positive potentials are restricted to the culmen. Late activity is very widespread in this experiment which should be contrasted with the more usual distribution of activity as shown in Fig. 2 A.

leading from the pyramis, folium and tuber vermis and uvula. Potentials were never absent on the pyramis and present on any other lobule.

The latency of the response as measured from the time the tendon was struck to the beginning of the surface positive deflection on the pyramis was

MATERIAL AND METHODS

The present study is based upon observations on 34 rats. They were lightly anesthetized with nembutal, 0.1 cc. of a 6 per cent solution per 250 g. Methods of stimulation were chosen which would give a fairly synchronous volley of impulses. For the proprioceptive stimulation the quadriceps femoris muscle in the hindlimb and the triceps brachii muscle in the forelimb were detached at their insertion and fastened to the animal board by a taut cord. The femur or humerus was fastened securely by a small specially made clamp and the remainder of the limb frequently denervated. Care was always taken that sufficient skin was removed from the extremity so that tapping the cord which suspended the muscle caused no movement of any part of the animal except the muscle itself. The cord was struck lightly, by a small metal rod attached to an electro-magnetic device which was activated at the proper sequence of each sweep of the beam of the cathode-ray oscillograph. This produced a synchronous excitation of stretch receptors. For the exteroceptive stimulation an ordinary wooden applicator was fastened to the above device so that it would sweep parallel to the surface of the animal's body in a cephalic direction but only close enough to move the hair of the back or proximal parts of the limbs. The skin itself was never touched nor was the slightest movement of the animal's body produced. In an active preparation only the tips of the longest hairs need be touched by the moving applicator to produce an action potential on the cerebellum. The amount of excursion of the device could be adjusted so that if desired a very localized patch of hair could be stimulated. In some experiments where the latency of the response was determined a "click" sound was produced by the electro-magnetic device. Although the presence or absence of this sound affected the action potentials led from the inferior colliculus no effect was ever seen on the cerebellar responses, nor was the "click" itself ever observed to produce any cerebellar response.

The lead electrode was usually a pointed cotton wick moistened with Ringer's solution and applied lightly to the exposed surface of the cerebellum. An indifferent electrode was applied to the skull. In a few experiments steel needles, insulated except at their tips, were used as lead electrodes. These were mounted in a holder 1 mm. apart. In no instance did the lobes giving responses with these "bipolar" electrodes differ from the lobes giving responses when explored by the single cotton wick electrode. Only the surface lobes which could be exposed readily were studied. These included the highest folium of the culmen, the lobulus simplex, the folium and tuber vermis, the pyramis, the uvula, Crus I and II of the lobulus ansiformis and the lobulus paramedianus. The basal parts of the anterior lobe, the paraflocculus and the flocculonodular lobe were not explored. In some experiments simultaneous records were taken from two points on the cerebellum and the responses from an identical stimulus compared. The action potentials were amplified by condensor coupled amplifiers and recorded by a cathode-ray oscillograph.

RESULTS

Stimulation of quadriceps femoris muscle. Cerebellar responses following stimulation of proprioceptive endings in the quadriceps muscle were studied in 18 animals. In seventeen of them potentials of significant amplitude were obtained when leading from the cerebellum. In twelve of these experiments the responses of greatest amplitude were recorded when the leads were on the pyramis, Fig. 1B. The potentials recorded from the pyramis were always surface positive while those from the other lobes explored were usually surface negative or diphasic. When surface positive potentials of large amplitude were lead from other lobes besides the pyramis they invariably appeared after a much longer latency than the pyramidal response. The most surprising finding was the almost complete absence of potential change when leading from the uppermost folium of the culmen. The deeper folia of the culmen were not explored. In certain experiments all the other lobules explored showed some activity. In some experiments responses were found only when

the quadriceps were relatively long the triceps responses were detected about 3 msec. earlier than those resulting from the stimulation of the quadriceps.

Stimulation of hair. These experiments were done on 28 animals, 14 of which were also used for studying the effects of proprioceptive stimulation. The responses on the cerebellum resulting from this type of stimulation were more variable in their distribution, their sign and duration than was the case for either proprioceptive stimulation or electrical stimulation of peripheral nerves. In 8 experiments responses were detected on all the lobules of the rat's cerebellum, which we explored (Fig. 1, D). In 26 out of 28 animals the culmen showed the largest response and in many the only surface positive potential. In about half of the experiments a small surface positive potential was also seen on the lobulus simplex (Fig. 2, B). These deflections, whose duration was 15 to 20 msec., were usually followed by a long surface negative potential which lasted 50 to 75 msec. In some preparations (Fig. 2, B) this was practically the the only activity seen. Usually if any potential was seen coincident with the positive potential on the culmen it was a surface negative deflection (Fig. 1, D; Fig. 2, B). This m'ght in turn be followed, in active preparations, by a late surface positive or surface negative potential. Of particular interest was the contrast between the responses on pyramis and uppermost folium of the culmen when exteroceptive and proprioceptive stimulation was

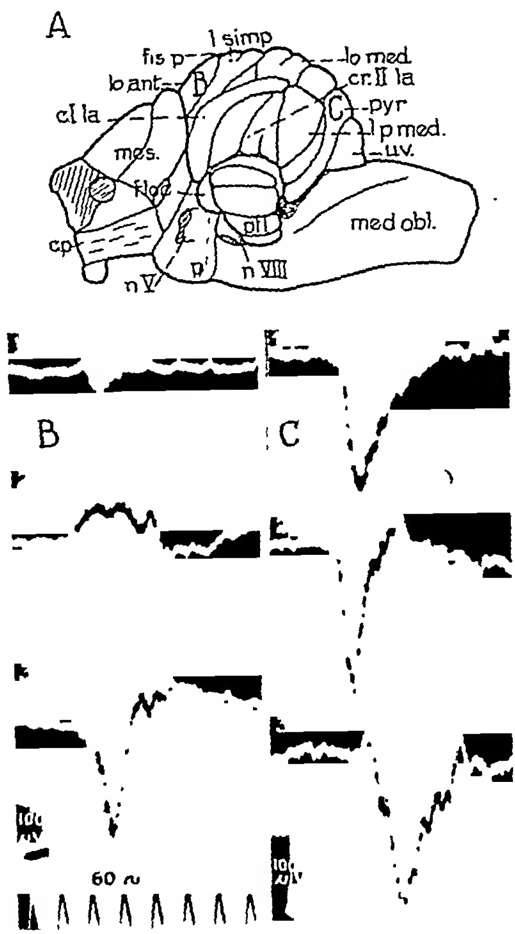

FIG. 3. A. Lateral view of the rat's cerebellum. Letters B and C indicate the lead points for their respective records. B. Records with lead on the highest folium of the culmen. Stimuli applied without changing lead electrodes. 1, tendon of the homolateral quadriceps femoris tapped; 2, tendon of the homolateral triceps brachii tapped; 3, hair on the animal's back moved. Stimulus artifacts are not shown but the records are mounted so that time sequence of each pair correspond. C. Simultaneous records led from the pyramis following the identical stimuli which produced those in B. Note that although a surface positive potential of considerable size is present in the pyramis record following stimulation of the hair, C3, it occurs 16 msec. after the culmen response.

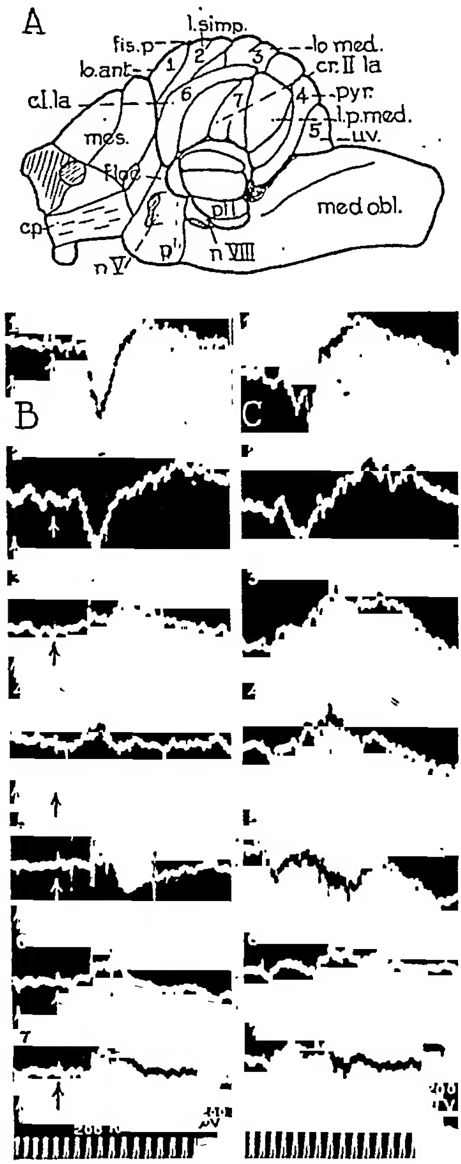

FIG. 2. A. Lateral view of the rat's cerebellum. Nos. 1 to 7 refer to the lead points for the corresponding records. B. Potentials recorded following stimulation by moving the hairs on the animal's back. The first arrow indicates the artifact caused by the electrical impulse which activates the electro-magnetic device. The beginning of actual stimulation is 5 msec. later. The second arrow indicates the end of stimulation. The deflections of short duration are artifacts caused by the electro-magnetic device. Note the early surface positive potentials are found only on the upper folium of the culmen, B1, and the lobulus simplex, B2. C. Same lead points with single shock electrical stimulation of the homolateral sciatic nerve.

usually between 5 and 8 msec. This is significantly less than the figure given in the preliminary report of this work (5) but a more accurate method of determining the instant of stimulation eliminated a source of error which was responsible for the incorrect figure of 16 to 18 msec. previously given.

Stimulation of triceps brachii muscle. The triceps muscle was stimulated in 12 of the above 18 experiments and in two additional animals. In all but two experiments the responses of greatest amplitude were found when leading from the pyramis. These two exceptions were hyperactive preparations and in these animals potentials of greater amplitude were found on the lobulus ansiformis (Fig. 1, C7). The peaks of these potentials occurred 15 msec. after the peak of the potential recorded from the pyramis. In many of the experiments responses were confined to the pyramis, uvula and folium and tuber vermis. Indeed the only difference in the areas showing responses when the triceps was stimulated as compared to those when the quadriceps was stimulated was the occasional appearance of potentials on the culmen when the triceps was stimulated (Fig. 3, B2). Differences in latency were not always demonstrable between the responses from stimulation of the triceps and quadriceps muscles. In experiments in which the latencies from

potentials actually originated, decerebration and destruction of the inferior colliculus or the cerebellum was carried out at the end of many of the above experiments. Complete removal of the cerebellum always eliminated the responses on the inferior colliculus to movement of hair (Fig. 4, e). The removal of the cerebellum did not itself damage the colliculus. This could be shown by the fact that responses to "clicks" could still be detected (Fig. 4, d). Removal of the colliculus or decerebration at the level of the inferior colliculus did not interfere with the cerebellar potentials as long as the general condition of the animals did not deteriorate. Potentials identical in appearance to those found on the colliculus following exteroceptive stimulation could be detected if the lead electrode was applied to a pledget of cotton placed in the site of the extirpated inferior colliculus. It is our belief that all potentials recorded from the inferior colliculus following movement of the animal's hair originated in the anterior lobe of the cerebellum.

Electrical stimulation of sciatic nerve. Electrical stimulation of the sciatic nerve in the rat (Fig. 2, C), produced more widespread responses on the cerebellum than was the case in the cat (2), and much more so than in the monkey (3). Although it might be expected that electrical stimulation of a mixed nerve like the sciatic would give the sum of the responses produced by the proprioceptive and exteroceptive stimulation such was not exactly the case. In fact much to our surprise the distribution of the potentials resulting from this stimulation resembled closely that produced by moving the hair. Although in a given experiment responses to electrical stimulation might be of greater relative size on the pyramis than with exteroceptive stimulation (Fig. 2, B4 and C4), in none of the 5 animals in which the sciatic was stimulated electrically were action potentials found when leading from the pyramis which were as large as those produced by tapping the tendon of the triceps or quadriceps muscles. Whether or not this observation will be confirmed in a large series of animals remains to be seen. The responses in the vermian lobes began 5 msec. after the shock artifact. As was true of both exteroceptive and proprioceptive stimulation, late responses on the ansiformis were occasionally seen following electrical stimulation. When these were present they began 20 to 30 msec. after the shock artifact.

Discussion

A volley of impulses conducted toward the surface of the cerebral cortex is evidenced by an action potential of positive sign when a single lead is applied to the surface (1). If this is true for the potentials described above it would appear that of the cerebellar lobes explored in these experiments, the richest and most direct projection of fibers which may be activated by stimulation of proprioceptives is to the pyramis. Similarly, the culmen would represent the site of termination of most of the afferent fibers to the cerebellum which are activated by exteroceptive stimulation. These differences in the distribution of the early surface positive responses raises the possibility that the spinocerebellar tracts may differ in the modalities of sensation which each carries to the cerebellum.

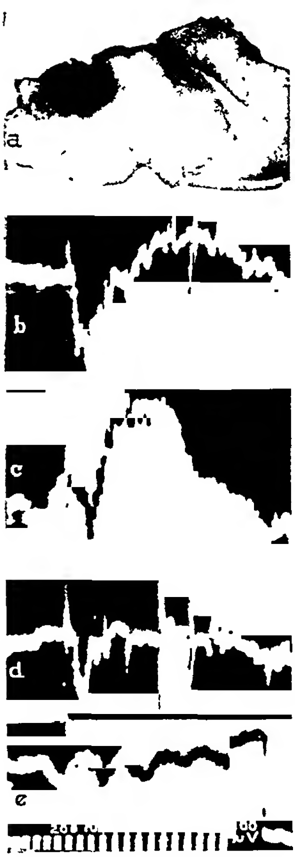

FIG. 4. a. Photograph of the mid-sagittal section of the rat's cerebellum following extirpation of all the cerebellum except the most caudal folium. The space ordinarily occupied by the central part of the cerebellum is filled by a blood clot. b. Response on the highest folium of the culmen following moving the animal's hair. c. Lead from the inferior colliculus before ablation of the cerebellum, with stimulation as in b. "Spontaneous" activity in the colliculus distorts the response. d. Lead from the inferior colliculus after ablation of the cerebellum; stimulation as in b. The movement of hair for the records b, c, and d was accompanied by a "click" sound which occurred at the arrow. Note the presence of a surface positive potential shortly after this sound stimulation. Same response was obtained with "click" alone without touching the hair. e. Same lead and same exteroceptive stimulation but without sound. Note the absence of a response from the inferior colliculus.

given. As may be seen in Fig. 3, proprioceptive stimulation of either the fore or hind limb causes more activity in the pyramis than in the culmen when a comparison was made with identical stimuli. With stimulation produced by moving the hair either the reverse occurred or as was the case in this experiment (Fig. 3) both pyramis and culmen are activated. It should be emphasized however that when the latter takes place the peak of the action potential in the pyramis appeared appreciably after that in the culmen; 15 msec. in the experiment shown in Fig. 3.

The responses to stimulation of the exteroceptors did not differ in any consistent way when the hair on different parts of the trunk or proximal parts of either homolateral extremity was moved. The latency of the responses following exteroceptive stimulation was difficult to determine because the stimulus itself was not instantaneous. It occupied 5 to 15 msec., depending on how large an area of hair was moved. The surface positive potential on the culmen appeared 20 to 30 msec. after the beginning of the stimulation and reached its peak 7 to 10 msec. later.

The peak of the late potentials occurred from 55 to 75 msec. after the beginning of the stimulation. These late responses were much more frequent following movement of the hair than in the stimulation of proprioceptive endings. Whether they represented the arrival of a long delayed afferent volley to the cerebellum or were the result of intracerebellar connections was not determined.

Because of the close proximity of the inferior colliculus to the anterior lobe of the cerebellum in the rat and the frequent bleeding at this site, following exteroceptive stimulation, an action potential could frequently be led from the inferior colliculus (Fig. 4, c). In order to be certain where the

of the small size of its cerebellum, the rat is not the most suitable animal for this study.

4. In respect to the cerebellar lobules activated, electrical stimulation of the sciatic nerve resembled exteroceptive stimulation more closely than proprioceptive stimulation. The distribution of activity throughout the cerebellum following electrical stimulation of the sciatic nerve in the rat was more widespread than in the cat, and much more so than in the monkey.

The authors wish to express their thanks to Mr. Fred Claussen for technical assistance.

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Grundfest and Campbell (6) reported that electrical activity is initiated in the dorsal spinocerebellar tract by stimulation of nerves supplying muscles of the hindlimb but not by stimulation of the saphenous nerve. They found the cerebellar lobules activated by the dorsal spinocerebel ar tract to be in the basal folia of the anterior lobe. These folia are difficult to expose in the rat and it is not known whether they are activated by exteroceptive or proprioceptive stimulation in this species. A study by Marchi technique of possible differences in the termination of the dorsal and ventral spinocerebellar tracts in the rat is now being made. Snider and Stowell's (7) success in detecting action potentials in the cat's cerebellum in response to tactile stimulation opens the possibilities for further study of these differences in a species with a larger cerebellum and one more suitable for operative procedures on the cerebellar peduncles.

Our failure to find differences in the cerebellar action potentials depending on the topographical regions stimulated is not surprising in view of the fact that electrical stimulation (2) also failed to show any such differences. This is in accord with the lack of segmental differences in the termination of the spinocerebellar tracts as determined by experimental anatomical methods. (For a review of anatomical data bearing on this see 4.) However, the rat because of the small size of its cerebellum is probably not as suitable as some larger animal would be for the study of possible differences in the connections from topographically different parts of the body. Snider and Stowell (7) state that in the cat such differences exist and that it is possible to construct a map of the cerebellum showing the arrangement of the representation of different parts of the body. This is the first evidence of clear cut topographical localization in the cerebellum in respect to its afferent connections. If consistently confirmed it is an observation of first magnitude.

Summary and Conclusions

1. Cerebellar action potentials in the rat were recorded from surface folia following stimulation of exteroceptive receptors by moving the hair on different regions of the animal's body and of proprioceptive receptors by tapping the tendons of isolated muscles of the fore and hind limb. The lobules explored were the highest part of the culmen, the lobulus simplex, the folium and tuber vermis, the pyramis, the uvula, Crus I and Crus II of the lobulus ansiformis and the lobulus paramedianus.

2. Of the lobules explored the pyramis showed the most consistent activity following proprioceptive stimulation and the culmen following exteroceptive stimulation. There were marked differences in distribution of the responses depending on which type of stimulation was used. Some of the potentials may have resulted from activity caused by intracerebellar connections.

3. No consistent differences in the distribution of the responses was detected when different parts of the animal's body were stimulated. Because

A COMPARISON OF EFFECTS OF UPPER AND LOWER MOTOR NEURONE LESIONS ON SKELETAL MUSCLE

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THE "WASTING" of paralyzed muscles has been recognized since antiquity. It received mention in the Bible. John Hunter, in his Croonian Lectures (6), stated the fact as one generally familiar. Differentiation between paralysis due to spinal trauma and that caused by peripheral nerve injury was implied by Pott (7) in the description of the disease now bearing his name. Barlow (1) recognized the main characteristics of the two types of paralysis. Essentially modern views on the subject were current by 1884 when Bramwell (2) wrote, "Where the lesion is situated above—the multipolar nerve cells of the anterior cornu the paralyzed muscles may be—moderately wasted (atrophy of disuse). Where the multipolar nerve cells are acutely destroyed—rapid atrophy results."

In recent times there has been a considerable body of clinical observation covering this field. The experimental approach was relatively neglected until Tower (10), using dogs, demonstrated atrophy in muscles connected to an isolated section of the spinal cord. This part of the cord was severed from the rest of the nervous system by cutting the dorsal roots and performing a complete transection of the cord itself above and below the region involved. Later, Tower, Howe and Bodian (11) found that a similarly produced atrophy in monkeys was not accompanied by fibrillation. Eccles (4), using cats subjected to the same experimental procedure, investigated the contractile properties of such neurologically isolated muscle and confirmed Tower's findings with relation to the atrophy.

Using albino rats we (9) have demonstrated marked atrophy in hind limb muscles due to high-section of the spinal cord. Such section does not "isolate" the involved segment of the cord, as with Tower's technique, but does abolish the upper motor neurone control of the muscles of the lower limb. Tower's method produces neurological isolation of the muscles being studied while the simple cord section removes higher control but leaves the spinal reflex arcs intact. The present experiments were undertaken to compare atrophy, fibrillary activity and acetylcholine sensitivity of muscles deprived of their upper motor neurone control with these properties in muscles deprived of lower motor neurone control, the so-called denervated muscles.

METHODS

Albino rats weighing between 200 and 350 gm. were used throughout. They were

* Aided by a grant from The National Foundation for Infantile Paralysis, Inc.

weight. The estimated atrophy was probably less than actually took place, as there was a general loss of body weight. However, the progressive and parallel courses of the atrophic processes are apparent from this graph. For comparison, the course of atrophy following denervation in otherwise normal animals is included in the figure. Here, of course, D and I are actual determinations of the weights of the denervated and normal muscles.

Between the second and third weeks after operation a change became apparent. The animals continued to drag their hind limbs, remained incontinent, and appeared generally comparable to those sacrificed earlier, which led us to believe that no effective repair of the cord lesion had taken place.

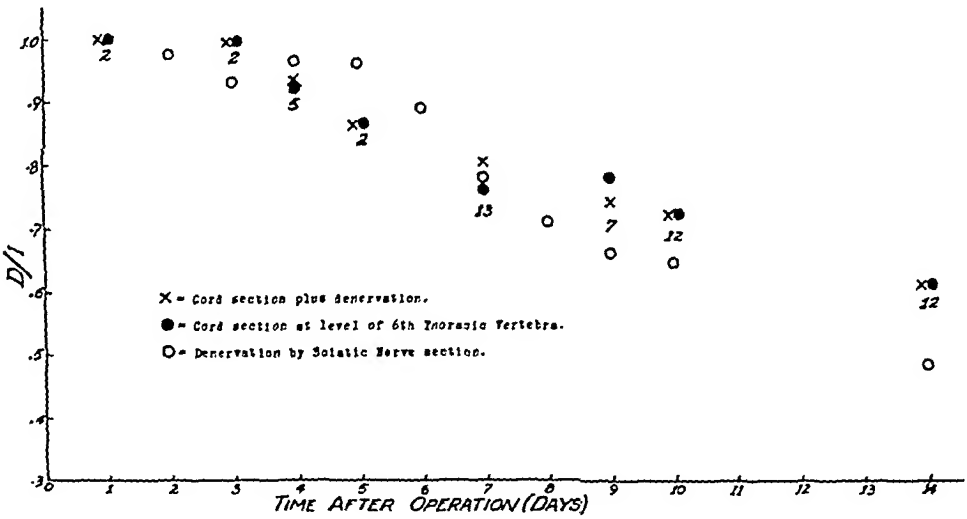

FIG. 1. Muscle atrophy following the operation indicated in the legend. The figures beside the points indicate the number of animals in the "Cord section plus denervation" and the "Cord section at level of 6th thoracic vertebra" series.

However, as time went on, a difference in the size of the muscle-groups on the two sides became increasingly evident. The weight loss progressed in the denervated muscles, whereas recovery took place in the plegic side. In Fig. 2, the time scale of Fig. 1 has been reduced and the duration increased to show graphically the course of this divergence. The plegic muscles, deprived of upper motor neurone control, recovered rapidly from the initial marked atrophy and, in the longer experiments, almost regained their normal size and appearance. In the denervated muscles, however, atrophy advanced steadily until, at the end of 8 weeks, little more than thin bands of contractile tissue remained.

The muscle groups of several animals were examined histologically in paraffin sections. In the first 2 weeks, both showed a shrinking of muscle fibres and proliferation of sarcolemmal nuclei but the fibres retained their striations. Later, however, the fibres of the plegic muscles recovered a nor-

maintained in separate cages in a well-heated room at a fairly constant temperature. An adequate diet was available to all animals. Under ether anaesthesia the vertebral column was exposed and complete section of the spinal cord performed at about the level of the 6th thoracic vertebra. At the same time, denervation of the right gastrocnemius-soleus muscle group was effected by removal of a length of the sciatic nerve on that side. In animals which were to be kept for long periods of time, the completeness of this denervation was checked during the 3rd or 4th week.

In the initial experiments the immediate mortality, and that during the first few post-operative days, was high. This was lowered appreciably, however, by maintaining the animals in a room kept at a fairly constant temperature (about 80°F.). The animals appeared normal anterior to the mid-thoracic region but, of course, were incontinent and dragged their lower extremities. When spinal shock had passed off, *i.e.*, within a day or two, the lower limbs on the two sides revealed gross functional differences. The right was limp, whereas the left showed an immediate mass flexor reflex response when it was pinched but appeared relaxed except when thus stimulated. At various intervals after operation the animals were sacrificed, the fresh weights of both gastrocnemius-soleus groups determined, and each animal weighed.

In most cases, just prior to autopsy, the animals, under light ether anaesthesia, were subjected to the following procedure. With the limb immobilized, the tendon of the muscle group of one or other side was dissected out, detached from its insertion, and attached to an isotonic lever for recording on a smoked drum. In many but not in all cases the intact sciatic nerve was severed at this time to abolish reflex movements and thus simplify the experimental procedure. The action potentials were led from the muscle belly through two fine needles and amplified. They were recorded photographically, using a Matthews oscillograph, and rendered audible through a loudspeaker. Injections of acetylcholine in various dilutions but constant volumes (0.25 cc.) were made into the iliac artery, and the minimal effective dosage determined. Normal control animals of comparable weights were subjected to the same procedure.

Results

In order to simplify description, the muscle or limb on the right side will be referred to as "denervated," that on the left side as "plegic," and those in a normal control animal as "normal." The word "plegic" is derived by deleting the prefix "para," inapplicable in this case, from the familiar term "paraplegic." It should be noted that the so-called "plegic" muscles showed no evidence of activity except as a reflex response to noxious mechanical stimulation or as a direct response to other forms of stimuli.

1. *Atrophy.* In all, 83 animals were used and killed at various intervals after operation. Those sacrificed in the first two or three weeks had lost considerable weight, but the later animals showed a subsequent gain.

During the first two weeks, the gastrocnemius-soleus muscle groups in any one animal showed practically identical weight loss on the denervated and plegic sides. Differences up to 10 per cent were encountered between the weights of the muscles on the two sides in occasional animals, but such deviations showed no consistency in either direction.

Figure 1 shows graphically the course of muscle weight loss in these animals. As in our previous paper (8), atrophy is represented by the decimal fraction of the ratio D/I. D is the fresh wet weight of the atrophic muscle, and I the weight of the corresponding normal muscle. In the present series, an estimation of a value for I was made on the basis of the animal's body weight at autopsy. This seems justified as, in a large group of normal animals, the gastrocnemius-soleus muscles bore a fairly constant ratio (1:65) to body

3. *Acetylcholine sensitivity.* Brown (3) stressed the marked sensitivity of denervated skeletal muscle to the intravenous or intra-arterial injection of acetylcholine. In the rat, it has been shown that the denervated gastrocnemius-soleus group shows a minimal response to the intra-arterial administration of doses of 0.002–0.02γ of this material. On the other hand, only dosages of 200γ–2.0 mg. were effective in stimulating comparable normal muscles. In the present experiments, a similar comparison was made between the denervated and plegic muscles at various stages in the course of atrophy depicted in Fig. 2. For the first 2 or 3 days, the muscle groups on both sides behaved as normal muscles in response to acetylcholine injections. The lower record of Fig. 3, which shows the response of such a normal muscle, is the only one of 30 experiments where 200γ of acetylcholine produced any mechanical movement. In the remainder, 2 mg. doses were necessary to be effective.

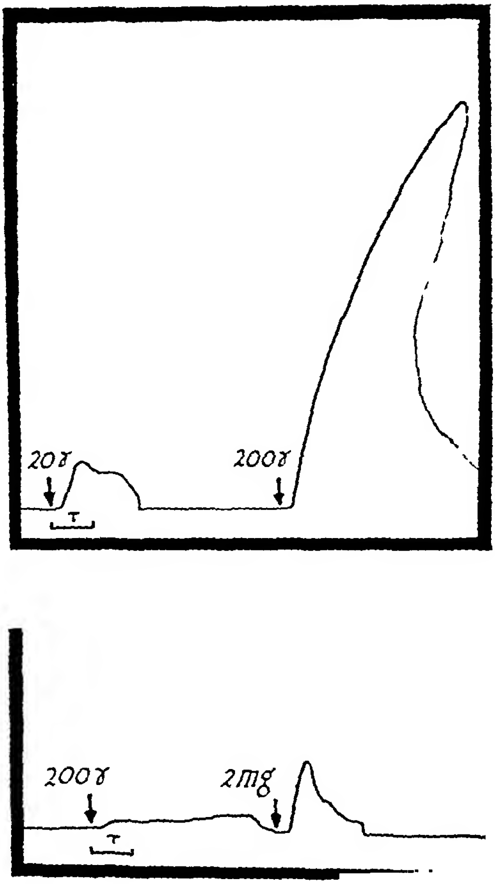

Fig. 3. Mechanical responses of gastrocnemius-soleus muscles of rat to intra-arterial injections of acetylcholine at points indicated by arrows.

Upper record shows the reaction of muscle with upper motor neurone lesion to injections of 20γ and 200γ of acetylcholine.

Lower record shows the reaction of normal muscle to 200γ and 2 mg.
T = 10 sec.

Commencing at the third day after operation, however, the denervated muscles showed an increasing sensitivity to acetylcholine which was at its maximum by the 6th or 7th day. These muscles were stimulated by doses of 0.002–0.02γ administered intra-arterially. It was difficult, in the animals tested several weeks after operation, to be certain of minimal mechanical movements in the presence of such marked atrophy. However, it appeared that even after 8 weeks this increase in sensitivity to acetylcholine was still present.

Again commencing at the 3d day, a much smaller, but nevertheless definite, change in sensitivity was exhibited by the plegic muscles. Approximately 20γ of acetylcholine produced a response in this group of muscles. Not only were the muscles stimulated by doses which were thus 10–100

mal histological appearance, whereas the denervated muscles progressed to typical atrophy. Good nerve endings were readily demonstrable, with gold technique, in the plegic groups, whereas repeated attempts failed to demonstrate any such endings in the denervated preparations.*

2. *Action potentials.* When these animals were tested at any time after the 3d postoperative day, the typical action potentials of fibrillation were readily detected on the denervated side. These were at a maximum between

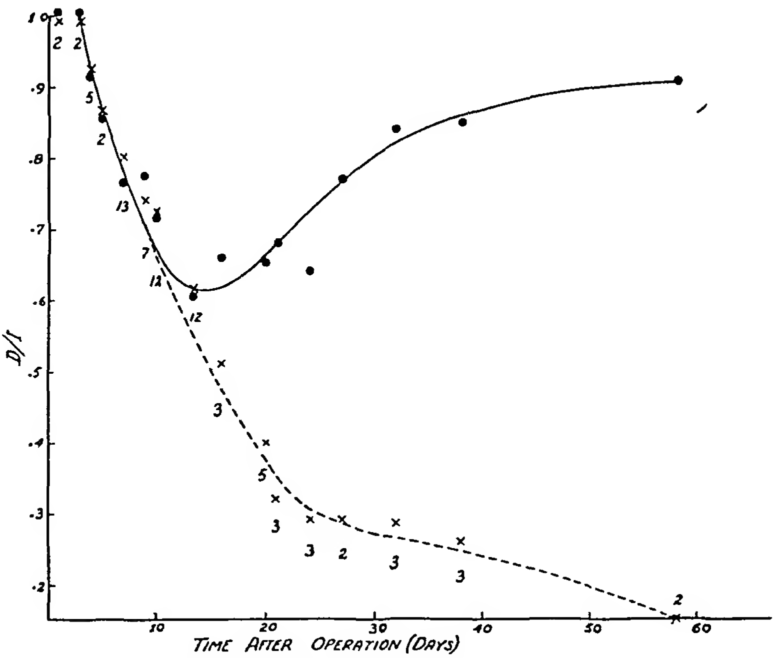

FIG. 2. An extension of Fig. 1 showing muscle atrophy following "Cord section at level of 6th thoracic vertebra" (•) and "Cord section plus denervation" (×). The figures besides the points indicate the number of animals contributing to the averages represented by the points.

the 6th and 14th days, but subsequently decreased in amplitude. After 8 weeks, though much diminished, they were still detectable. These action potentials of the denervated muscle were not modified by even vigorous pinching of the limb, but were readily abolished by quinidine (8).

On the plegic side, under the light ether anaesthesia, no activity was observed at rest. In response to mechanical stimulation, an immediate outburst of action potentials resulted. This outburst, the electrical concomitant of the mass reflex response described above, could be elicited 2 days after operation and was detectable even 8 weeks later. It could be abolished by section of the sciatic nerve but was unaffected by doses of quinidine which would abolish the fibrillation of denervated muscle.

* We are indebted to Professor E. A. Linell and Dr. M. I. Tom of the Department of Neuropathology for this examination.

weight loss and one would expect to detect fibrillation. The plegic muscles always showed regression of atrophy and never exhibited fibrillation. Furthermore, if anterior horn cell damage were the cause of the atrophy a cordotomy performed several segments lower should prolong the atrophy or render it permanent. The onset and regression of atrophy were unchanged when this was done.

The plegic muscles were apparently, apart from occasional reflex spasms, at rest. They showed no prolonged spastic state detectable by our methods and were certainly not subjected to as regular a bombardment by motor nerve impulses as are normal muscles. Disuse, relative or absolute, appeared to be a constant factor throughout the whole course of the experiments as judged by the behaviour of the animals. It is possible, however, that there was a low level of reflex activity in these muscles producing a slight steady contraction not noted on manipulation. Such an assumption in no way helps to explain the bizarre course of the atrophy.

Perhaps the onset and subsequent regression of atrophy in the case of an upper motor neurone lesion and onset and subsequent accentuation of atrophy in the case of a lower motor neurone lesion indicates the activity of two etiological processes. It is possible that the first short-lived process is seen in action in both types of damage but the second is active or is unopposed only in the case of denervated muscle. Knowledge of the cause of the regression of atrophy in the case of an upper motor neurone lesion may yield the key to effective treatment of denervated muscle.

The experiments of Tower (10, 11) and Eccles (4) in which muscles are left with an intact lower motor neurone but are protected from motor nerve impulses by isolation of the involved section of the spinal cord probably differ fundamentally from our experiments. In the one case the muscle is neurologically isolated and in the other the reflex arc is intact and the muscle is subjected to occasional large bursts of impulses producing massive contractions, possibly also, as previously suggested, to a steady flow of impulses producing a level of constant activity too small to be detected by our methods. Neither Tower nor Eccles attempted to follow the course of atrophy in their experiments and it is impossible to tell whether their animals showed the onset and regression of atrophy seen after simple cord section. In both types of experiments pyramidal (upper motor neurone) and extrapyramidal motor tracts are cut. While the former are predominant in their effect on muscular activity it is possible that simultaneous section of the latter modified the atrophic process. However, we know of no clear-cut evidence, either experimental or clinical, indicating that spinal cord tracts other than the pyramidal have a trophic influence on skeletal muscle.

In the present experiments a response in the muscles with intact motor nerves was elicited by smaller doses of acetylcholine than in normal muscles. However, this increased acetylcholine sensitivity was only seen during the period of atrophy. As recovery commenced, the muscles showed return to a normal degree of excitability. In the case of the denervated muscle greatly

times lower than those necessary for normal muscles, but, as seen in Fig. 3, larger doses produced responses of greater magnitude than were ever seen with normal muscles. It was suspected at first that these results might be explained by destruction of some anterior horn cells in the initial operative procedure. In this event a partial denervation of the muscle might have been produced without being extensive enough to produce fibrillation detectable by our technique. However, cordotomy at levels well above and below the site usually chosen produced no fibrillation, and the increased sensitivity of the plegic muscles was unchanged. These findings indicated that it was most improbable that the lowered threshold to excitation by acetylcholine was caused by undetected lower motor neurone damage. Furthermore, this altered threshold only persisted during the first 2–3 weeks postoperatively. In animals tested after the 20th day, it was found, with 2 exceptions, that the spastic muscles responded only to the larger doses which were effective with normal muscles (2.0 mg.). The exceptions were found on the 24th day (Fig. 2) when, in two of three animals tested, the plegic muscles showed the lower threshold and increased response to acetylcholine described above. It is to be noted that the atrophy of these particular muscles was a good deal more marked than one would have expected.

Discussion and Conclusions

In the experiments reported in this paper, the muscles of the lower limbs were deprived of normal upper motor neurone control by the transection of the spinal cord in the mid-thoracic region. This was modified by cutting the right sciatic nerve, in this way producing lower motor denervation on that side. Such animals showed an intact reflex arc and normal motor nerve endings on the left side. On the right side the denervated muscles demonstrated the typical changes of nerve degeneration with muscle fibrillation. In the first 2 weeks postoperatively, there was an equal and parallel atrophy in the gastrocnemius-soleus muscle groups of the two sides. Between the second and third weeks, a divergence appeared. Whereas the denervated muscles continued to lose weight steadily, those with intact motor nerves began to show a gradual recovery of muscle bulk which progressed until, in some cases, an approximately normal muscle weight was attained. Following the preliminary report (9) outlining this development and regression of atrophy Fischer (5) described experiments supporting these findings.

Throughout our experiments the animals presented the same general appearance, dragging the lower half of the body and remaining incontinent. Furthermore, the readily-elicited mass reflex response on the left side remained unchanged and there was no apparent increase in muscular activity to account for the regression of atrophy. Were the atrophy the result of a temporary damage to some or all of the anterior horn cells at operation, it would seem unlikely that the mass reflex would remain unchanged throughout the whole course of the experiments. Operative damage to such cells, if of any extent, would produce a permanent, rather than transient, muscle

STIMULUS FREQUENCY AS A MEANS OF ANALYZING SYNAPTIC ACTIVITY

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CONSIDERING that many sense organs activate the centres by long trains of impulses it is surprising how little is known about the central effect of this mode of stimulation. In this laboratory Bernhard and Skoglund (2) have shown that a transient reflex pattern can be evoked in cats by restricting stimulation of certain nerves (*e.g.*, the popliteal) to certain frequencies. It consists of late widespread extensor contractions in all limbs, rise in blood pressure, pupil dilatation, etc., is absent below frequencies of 50 per sec., maximal around 100 per sec., lasts some 20–30 sec. and then disappears despite continued stimulation, even at optimal frequency. Their result shows that stimulus frequency may be a means of selecting certain reflex patterns and is of interest in view of the fact that this frequency range is well within the limits of normal sense organ activity.

Gasser's laboratory (9, 13, 10) has supplied us with important information about the effect of stimulus frequency upon peripheral nerve, and Lorente de Nó and Graham (20) have shown that the summation of subnormality known from peripheral nerve and augmented by repeated stimuli, is of significance in the recovery cycle of motoneurons. These studies have drawn attention to the depressing effects of frequency, analogous in peripheral nerve and centres, but there must also be slow facilitating effects to account, for instance, for slow "recruitment" (16).

Depression of synaptic activity, "recruitment" and selective effects of frequency are phenomena which also have turned up in this work, which is devoted to preliminary observations on the effect of stimulus frequency on direct and relayed waves caused by stimulation with electrodes buried in the spinal cord when the response is recorded from the sciatic nerve (22, 17). Prior to analyzing the effect of long trains of impulses under separately evoked facilitating and depressing influences it was deemed necessary to possess some information about the effects of frequency as such on direct and relayed waves.

METHOD

Stimulating electrodes have been a pair of needles, at a distance of 2 mm., insulated except at the tip, thrust into the spinal cord of decerebrate cats through the *unopened* dura in the lumbal region, generally in a ventro-lateral position, sometimes in the dorsal root region. The sweep circuit operating through an amplifier has been connected through a compensator bridge to these electrodes. A maximal rate of 850 per sec. could be obtained by these means. The strength of the stimuli was independent of rate.

In most experiments the leads were on the sciatic nerve, one of them on the crushed end, and connected to a balanced condenser-coupled amplifier.

The sweep circuit drove the beam of the cathode ray horizontally at each stimulus and the film was passed slowly at right angles to the sweep. Now and then the film was

increased sensitivity to acetylcholine appeared early and remained throughout the course of the experiment. These findings indicate that atrophy accompanied by increased sensitivity to acetylcholine may be produced by upper as well as lower motor neurone lesions and suggest that this picture is not exclusively a result of motor nerve ending or lower motor neurone destruction.

Summary

Using albino rats the atrophy of the gastrocnemius-soleus group of muscles following an upper motor neurone lesion (produced by spinal cord section at the 6th thoracic segment) was compared with the atrophy following a lower motor neurone lesion (section of the sciatic nerve). The two types of lesions produced atrophic processes which were quantitatively similar for the first 14 days. Thereafter the muscle groups with severed upper motor neurones started to recover lost weight while those with lower motor neurones cut showed an uninterrupted development of atrophy. Both types of injury produced hypersensitivity to acetylcholine in the muscles involved. This lasted only as long as the atrophic process was active and was most marked in the cases of section of the lower motor neurone. Fibrillation was seen only when the lower motor neurone was cut.

Possible causes of the onset and regression of atrophy in skeletal muscle deprived of upper motor neurone control are discussed.

It gives us pleasure to acknowledge the interest taken in this work by Professor C. H. Best.

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five stimuli. Using this criterion the relayed wave with the central reflex time 4.8 msec. possesses the limiting frequency 100.

In the next record C, at stimulus frequency 140 per sec., only the direct and the first relayed wave are seen, further apart now, while this frequency means that the sweep traverses its path in 7.15 msec. as against 14.3 msec. in record A. The width of the sweep path in msec. is obtained from the inverse value of the frequency. In record D the frequency is 198 per sec. The first relayed wave still persists though now pushed near the end of the

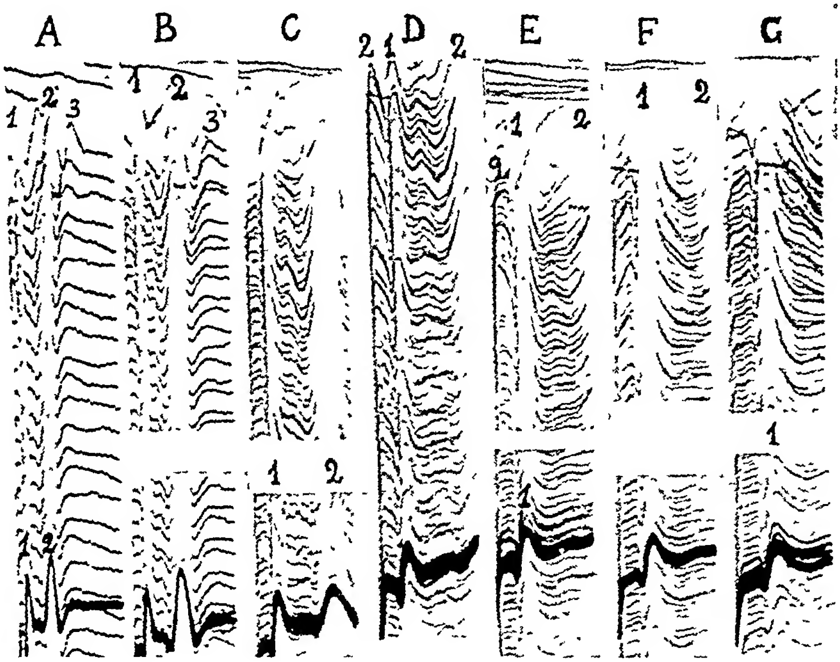

FIG. 1. Leads on the sciatic nerve. Bipolar stimulating needle electrodes in ventrolateral position 3 cm. above the cams of the cristae. Beginning of each record shown for different stimulation frequencies. Later in each record the film has been stopped to show some waves superimposed to give a good print. Full description of experiment in text. To be read downwards. In this and all records, 1, direct wave, 2 first, 3 second relayed wave.

picture. Its latent period is shorter in the beginning of the record and lengthens downwards. In record E, at frequency 230 per sec., the first relayed wave is present only in the beginning. In record F the frequency is 268 per sec. and this is the limiting frequency for the first relayed wave with central reflex time 2.1 msec. which is still seen in a few initial beats. In the last record G the frequency is 310 per sec. Only the direct wave is seen. At this frequency the greater part of the relayed wave would have passed over the shock artifact to reappear in the beginning of the sweep, if present. In records D, E, and F it is seen to have done so.

The experiment shows that the limiting frequency is reduced by a

stopped and several records superimposed, so as to give an average picture of the effect. Rather slow speeds had to be used to save film. As the sweep was linear no time marking was necessary and the total width of each picture, being inversely proportional to stimulus frequency, measured time directly. As a consequence the events recorded are expanded horizontally by an increase in stimulus frequency.

The cat was surrounded with vapour from a layer of water covering the bottom of the screened animal-box heated from below. The rectal temperature was around 37.5–38.5°. The animal was undenervated with the exception of the nerve to be recorded from, as our intention was to allow a background of free play of impulses from the natural sources.

Results

1. *General description of response.* Confirming Renshaw (22) and Lloyd (17) we find the direct spike wave caused by stimulation of the ventral horn cells or their axons, succeeded by one or several relayed waves. The direct wave is easily recognized by measuring its latent period which is practically equal to the conduction time at alpha velocity (around 80–100 per sec.). Simpler still is to diagnose it by stimulating with a high frequency. As is to be expected from the work of Gasser (9) direct waves generally follow the whole range available in the stimulator (350 per sec.) whereas relayed waves disappear far below this value. By subtracting the latent period of the direct wave from those of relayed waves the central reflex times of the latter are obtained. These, as we shall see, vary from case to case. The necessity of having a direct wave has restricted us to stimulation of places giving such waves (17) or to crossed stimuli symmetrically placed on the opposite side of a place for which the direct waves have been measured previously.

By means of Fig. 1, a typical experiment will be surveyed. The recording electrodes are on the undivided sciatic nerve. Stimulation begins in A with frequency 70 per sec. After 20 impluses the film is stopped for a moment. Two large waves are then clearly seen; the first, the direct wave with an average latent period of 0.9 msec., the second a relayed wave with an average latency of 3.0 msec. The central reflex time of the relayed wave is thus 2.1 msec., assuming that it is conducted in the same fibres as the direct wave. But going back in the same record A upwards there can be seen a second relayed wave, best marked in the beginning and fading out as stimulation proceeds. Its latent period is 5.7 msec. Subtracting the direct wave makes the central reflex time of the second relayed wave 4.8 msec., still assuming it to be conducted by the fibres conducting the direct wave. This assumption is a necessary approximation.

Record B of the same figure corresponds to stimulation frequency 100 per sec. Both relayed waves are seen in the beginning, the first alone when somewhat later (bottom of record) the film is stopped for a moment in order to make a good print. This frequency is the limiting frequency for the second relayed wave. It is, of course, possible to push the limen somewhat higher. For practical reasons it is, however, easier to take as the limiting frequency a value for which in the beginning of each record there are still traces left of the wave to be analyzed, say, for the first three to

The first peak is the direct wave, as measurements show, but how about the second peak in this case? The frequency test showed it to be present at rates (600 per sec.) as high as to be above any values seen with other relayed waves. It is, at least at this stage of our work, safer to regard secondary waves of this character as direct waves delayed by slower conduction rates in the motoneurones concerned.

Evidence for dispersion of the relayed waves is found in most records (22, 17). They are nearly always wider than the direct waves meaning that the synapses themselves differ in kind or number, that the diameter and length of the pathways differ or that facilitation in the short proprio-

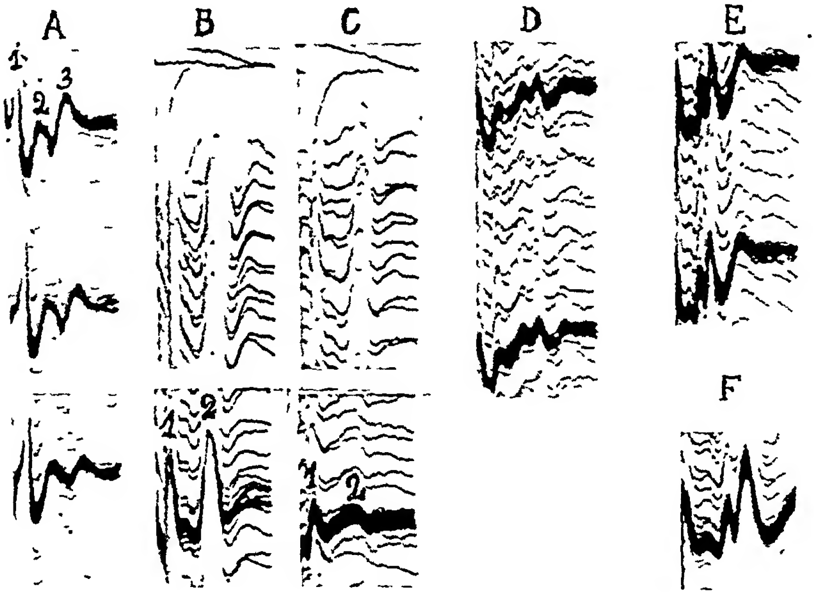

FIG. 3. A, leads on the peroneal nerve. Continued stimulation of spinal cord at 140 per sec. to illustrate typical decrease of relayed waves. B and C, leads on the sciatic in another experiment. Stimulation at 100 per sec. Stimulus strong in B, weak in C. Record D from another similar experiment shows the complex response often obtained when the stimulating electrodes have been placed on the contralateral side of the spinal cord. E and F from another experiment with the usual homolateral stimulation of ventrolateral side of spinal cord at frequencies 100 (E) and 198 (F) per sec. See text. To be read downwards.

spinal neurones (17) comes on slowly. The potentials set up by the simultaneously active and by the anatomical situation best synchronized synapses probably also activate adjacent elements electrotonically thus contributing to the dispersion. "Grouped action" of this type is very characteristic for motor neurones (23) and probably means a great deal for the characteristic grouping seen here. Nevertheless it is clear that the direct wave does not leave room for the same amount of temporal spacing around the top and thus represents stimulation of a more uniform system.

lengthening of the central reflex time and that, as stimulus frequency increases, the relayed waves disappear, so to speak, in good order and not by getting lost in general desynchronization. An essentially constant response pattern (but see below) is kept up until the limiting frequency is reached. During continued stimulation at higher frequencies the waves rapidly flatten out and disappear.

Figure 2 is introduced to show another type of pattern in which the direct wave is large and the first and second relayed waves are small because the stimulus is placed low in the cord. At slow speeds, A and B, the fast direct wave merely appears as a gap in the path of the sweep, but its size is shown late in record B when the film has been stopped for a while.

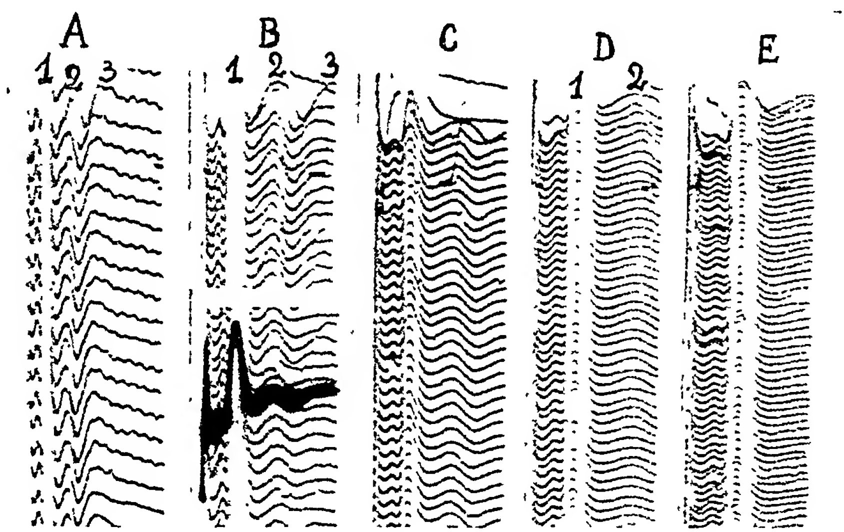

FIG. 2. Leads on the popliteal nerve. Bipolar stimulating needle electrodes just above the cams of the cristae. A, at 100 per sec.; B, 170 per sec.; C, 230 per sec.; D, 268 per sec.; E, 310 per sec. Limiting frequency between D and E, nearer to the latter. To be read downwards.

Figure 3A shows some portions of the course of the typical diminution of the two relayed waves of a response caused by repetition of the stimulus. The frequency is 140 per sec. This diminution which need not be noticeable for several seconds at lower frequency is also determined by the strength of the stimulus. Thus in Fig. 3, at 100 per sec., the stimulus is strong in record B and weaker in record C. The relayed wave decreases rapidly in C and not at all in B. The film is stopped after practically the same number of stimuli in both B and C, taken in succession with stimulating and recording electrodes in the same position. Record D of Fig. 3 illustrates the often relatively complex response of crossed stimuli to the spinal cord. It may, however, be much simpler. Records E and F in another experiment, taken at different rates of stimulation show the first wave to possess two peaks.

interesting point here is that the first and second relayed waves always vary in parallel, indicating a common influence on both of them, reminiscent of the intermittent conduction described by Barron and Matthews (1), rather than of the alteration seen at high frequencies in peripheral nerve. The latent periods of the three waves are here: 1.4, 2.9, and 4.9 msec. Their average latencies in several series were 1.3, 2.6, and 4.4 msec. Thus the average central reflex times of the first and second relayed waves were respectively 1.3 and 3.1 msec. corresponding to measured limiting frequencies of 340 and 200.

3. *Synaptic resonance.* Figure 5 begins with record A at a frequency of 160 per sec. There is a large direct wave with latent period 1.1 msec. It is followed by a very small relayed wave with latent period 2.3 msec. and a

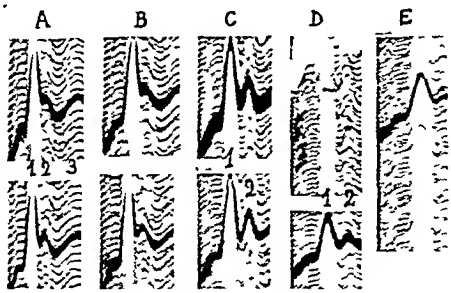

FIG. 5. Record A early and late portion of film, at 170 per sec., shows large direct wave immediately succeeded by just noticeable first relayed wave and broad second relayed wave. The first relayed wave is still small in B, at 198 per sec., but has greatly increased at stimulation rate 268 per sec. in record C. D at 310 per sec., and E at 360 per sec. Full description in text. To be read downwards.

second broad relayed wave at latent period 3.6 msec. The first relayed wave increases in size during stimulation in this and in the following record B at 198 per sec. In the next record C at stimulation rate 268 per sec. the first relayed wave is 4–5 times larger than in the beginning of the first record (A) and remains large for a considerable time during stimulation. Thus it has an optimum around this frequency range. The second relayed wave is still present in record D at 310 per sec. but on the verge of disappearing in record E at 360 which is the limiting frequency of this wave with an average central reflex time around 1 msec.

Certain relayed waves are often absent or questionable at slow rates of stimulation below 70 per sec. and turn up at higher rates again to disappear when the frequency increases. There are thus at times optimal rates of stimulation for certain relayed waves and in this sense true frequency resonance (2).

4. *Limiting frequency and central reflex time.* In correlating limiting frequency and central reflex time it is necessary to realize at the outset that such measurements cannot claim great accuracy. Several factors contribute to make precision unattainable. Latent periods are easier to measure with precision than limiting frequencies but partial overlapping of the waves in some cases and slightly increased latencies at faster rates of stimulation in others may make decision difficult. The average latent periods, leaving out the first 4–5 stimuli, have been used.

2. *Recruitment.* A relayed wave which is fully developed from the beginning generally decreases in size during continued stimulation (cf. Fig. 3), provided that the frequency be sufficiently high. But quite often relayed waves are encountered which actually *in*crease in size as a consequence of repetitive stimulation so that the total wave pattern is better developed after some time than in the beginning of a record. This result signifies that new neurones have been recruited into activity, partly perhaps also gradually improving synchronization. Lloyd (17) has studied the effects of facilitation to two or three shocks and also points out that the effect refers to grouped discharges with characteristic central reflex times.

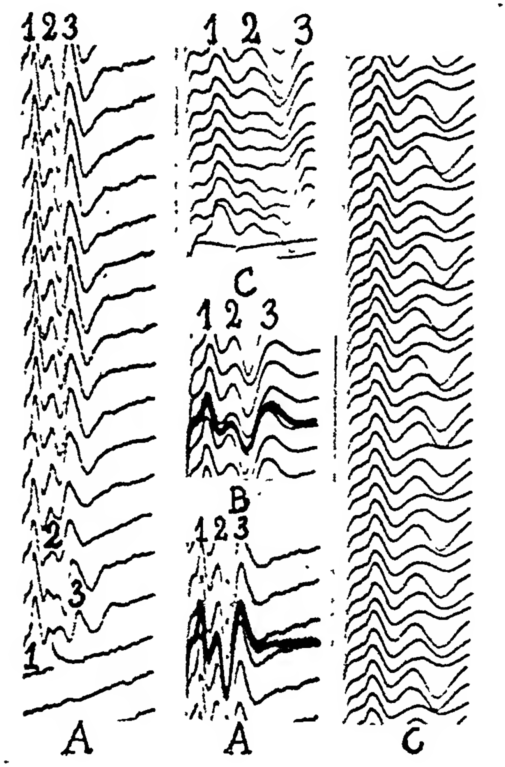

FIG. 4. All records to be read *upwards.* Record A, at 70 per sec. fully described in the text, continued into second row to show the fully developed effect with first and second relayed waves maximal and standing film. B, at 100 per sec. C, at 140 per sec. directly continued into third row. Full description in text.

Figure 4 illustrates a typical case. It begins with record A (to be read upwards) at frequency 70 per sec. In the beginning only the initial direct wave with latent period 1.0 msec., followed by the second relayed wave with latent period 3.8 msec., are clearly defined. Gradually a small wave becomes better visible between them. This is the first relayed wave with latent period 2.4 msec. The direct wave is of practically constant size throughout the record, but both the first and the second relayed waves increase in size up to about the 9th stimulus. At this size they were seen to remain during stimulation for a couple of seconds. A late portion of the film is found in the second row (A). Record B is around the 60th stimulus at rate 100 per sec. All three waves are clearly visible. Record C, continued in the third row, shows the beginning of the film taken at stimulation rate 140 per sec. The second relayed wave is now quite well developed from the beginning, but the first relayed wave is broad and not definitely set off from the rest until the 9th stimulus has been reached. The same record, continued in the next row, illustrates almost regular variations in the size of the relayed waves of the kind quite often seen at high frequencies. The

doubt is identical with the effect described by Graham and Lorente de Nó (13) and by them identified with the subnormality accompanying positive afterpotentials in peripheral nerve (10). As is well known from Gasser's work development of subnormality is greatly favored by rapid stimulation rates.

The shortest reflex times found in these experiments have been between 0.7 and 1.0 msec. Values below 1 msec. must refer to a single synapse (22, 17, 6). The maximal limiting frequencies have been about 360 per sec. Considering the rapid onset of subnormality with repetition, the absolute limiting frequency, defined as the capacity to pass only two volleys, would probably be a great deal higher. Alternation and intermittent conduction may also come in and complicate such determinations.

Discussion

The curves of Fig. 6 are interesting from two points of view, one eminently practical, the other theoretical. From the practical point of view it means that it may become possible to use curves of this type for analyzing the degree of complexity of the path between two points anywhere in the central nervous system, provided that the relation found is confirmed for other systems and that definite grouped discharges occur. So far we have only analyzed the relayed waves found in the dorsal columns (14) which have limiting frequencies falling around our curve. In this system there is also a direct wave which, of course, is necessary for establishing the form of the standard curve. But once this curve has been established for a given index in measuring limiting frequencies, the central reflex time can be located on it from measurements of limiting frequency alone, while the curve eliminates an important unknown factor, the datum provided by the direct wave. This can, of course, only be obtained for a relatively limited number of combinations of stimulating and recording electrodes.

When using the limiting frequency in this manner as an instrument for determining the central reflex time one may well ask whether it is possible to assume any fixed relationship between number of synapses and limiting frequency. Probably there exists some general relationship and probably number of relays is a decisive datum in determining a given point on the curve. But the dispersion of the relayed waves and the great variations indicate that other factors also play a rôle. The "delay path" (Forbes) responsible for a central reflex time of 5 msec. may, for instance, be determined by a minimal number of five synapses but delayed conduction owing to decreased fibre diameter and lengthened conduction distance are factors which hardly can be neglected and suggest some caution in interpreting the data. Still, we are inclined to hold that a *minimal* average number of 1 synapse per msec. is a reasonable assumption. The number may be greater but hardly less. It should also be realized that the first synapse occupies a favorable position compared with synapses placed later in the path on account of the more perfect synchronization of the stimuli for this synapse. This may be the reason why, as we have found (unpublished data), the

The limiting frequencies often interfere with the stimulus artifact and then may be difficult to determine. This is the main source of error especially at high frequencies. The relayed waves may be small or badly defined and we have no means of appraising strength of stimulation for a relayed wave, except in a very general manner. The limiting frequency in peripheral nerve, theoretically determined by the refractory period, is also determined by strength of stimulus and conduction distance (9) as well as by temporal dispersion caused by slowed conduction and increased latency (3). At higher frequencies it is necessary to use a brief period of stimulation and long intervals (9). These precautions have been observed. But the difficulties mentioned, in determining limiting frequency and others not particularly enumerated, all serve to emphasize that our curve, shown in Fig. 6, cannot do more than *indicate in a very general manner* the relationship between limiting frequency and central reflex time. There are also physiological factors causing variations when in other respects optimal conditions for measuring have prevailed.

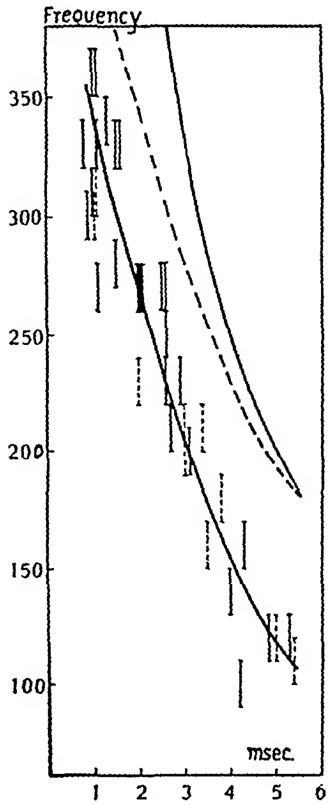

FIG. 6. *Curve on the left:* Limiting frequency of relayed waves (ordinates) plotted against central reflex times of same waves. *Curve on the right:* inverse values of central reflex times plotted against central reflex times. Curve *in broken lines:* curve to the left lifted upwards by a certain amount as explained in text.

In Fig. 6 data from crossed stimulation are marked by broken lines. It is at once seen that these data tend to be grouped in the lower half of the curve, whereas those taken from experiments with ipsilateral stimuli are grouped around the upper half of the curve. There are exceptions from this rule. But in general the central reflex times are longer for delayed waves elicited by crossed stimuli.

The upper curve is put in to illustrate the theoretical limiting frequency given by the inverse values of the central reflex times. The vertical distance between the theoretical and the experimental curve is, of course, also a function of the definition of the limiting frequency. The experimental curve could be pushed upwards by requiring only two shocks to be effective at a given frequency of stimulation, but practical reasons have necessitated a different choice of index, as pointed out above. But, if the experimental curve is lifted upwards to coincide with the theoretical curve at the lower end it can be seen that the two curves diverge for short latencies, meaning that the corresponding high frequencies possess a depressing effect augmented by stimulus repetition (cf. Fig. 1). This no

The relayed waves follow frequencies which decrease with increasing central reflex times. A curve illustrating limiting frequency as a function of central reflex time is given in Fig. 6.

Some relayed waves gradually recruit neurones and thereby increase in size, others have definite frequency optima and thus demonstrate the existence of synaptic resonance.

The findings are discussed in relation to excitation and inhibition and also with a view to suggesting use of the limiting frequency as a method of studying the central paths.

Variations in frequency as such can serve as a means of distributing into special channels effects entering a central path containing synapses.

The authors are indebted to the Rockefeller Foundation for support.

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motor end plate with a synaptic delay of 0.5 msec. (7) has much higher limiting frequencies than those found in the central nervous system, though in the latter the synaptic delay is of the same order (18, 19). A highly dispersed discharge as such would indicate complex pathways.

From the theoretical point of view the interest centers around the relation of these results to possible mechanisms of inhibition. Gasser's view that subnormality may be one such mechanism is borne out by the frequency effect upon the relayed waves activated by high frequencies capable of piling up subnormality very quickly. The frequencies concerned are within the physiological limits of the sense organs. For this type of inhibition McCouch, Hughes and Stewart (21) very properly suggest the use of Dusser de Barenne's term "extinction." But a new possibility is introduced by the fact that the delayed discharge caused by every single repeated stimulus is cut down from the "tail" end by relatively moderate frequencies. This suggests that long-synapse paths are particularly inhibitable merely *on account of the repetitive nature of the physiological stimulus. It also* suggests that inasmuch as the natural stimuli for some reason or other set up high frequencies the immediate effects are transmitted with preference into the synaptic channels of least resistance, *i.e.*, those with brief central reflex times. It is clear therefore that a frequency variation as such apart from how it may facilitate or block certain paths is a mechanism of significance in determining the central distribution of an effect entering a path containing synapses.

Late facilitation effects such as the recruitment and the synaptic resonance, described above, emphasize aspects of stimulus repetition which do not come out in the curve plotted as Fig. 6. Considering that in the superior cervical ganglion there is a late facilitation which has not required internuncial bombardment (4, 5); that this phase coincides with a slow negative potential change, and, further, that recruitment in peripheral nerve is favored by factors favoring the negative afterpotential (12, 8), it is probable that slow potential changes may serve to modify the central processes also in a positive direction. Synaptic resonance, demonstrated so clearly in the transient reflex discovered by Bernhard and Skoglund (2), is an interesting consequences of what different balancing influences may lead to.

Summary

When trains of electric stimuli are led through needle electrodes to the spinal cord certain wave patterns are set up which have been recorded from the sciatic nerve (decerebrate cats). These consist of a direct wave caused by stimulation of the ventral horn cells and by a number of relayed waves following after different latent periods.

By subtracting the latent period of the direct wave from those of relayed waves the central reflex times of the latter are obtained.

The direct waves follow frequencies of stimulation above 850 per sec.

The relayed waves follow frequencies which decrease with increasing central reflex times. A curve illustrating limiting frequency as a function of central reflex time is given in Fig. 6.

Some relayed waves gradually recruit neurones and thereby increase in size, others have definite frequency optima and thus demonstrate the existence of synaptic resonance.

The findings are discussed in relation to excitation and inhibition and also with a view to suggesting use of the limiting frequency as a method of studying the central paths.

Variations in frequency as such can serve as a means of distributing into special channels effects entering a central path containing synapses.

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THE CENTRAL EXCITATORY STATE ASSOCIATED WITH POSTURAL ABNORMALITIES

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Introduction

STRIATED muscle frequently exhibits varying degrees of rigidity in the absence of either voluntary contraction or organic neuromuscular disease. In the clinical literature this is usually called "spasm," yet there have been few analytical studies of this phenomenon. Jacobson (10) described what he termed "neuromuscular hypertonus," attributing the contraction in muscle to mental stresses.

Buchthal and Clemmeson (4) reported 46 cases in which electromyograms were made of "palpable muscle affections." They found "rest activity" in eight instances, and in a number of others, particularly where movement was painful, activity disappeared when the patient was arranged in a comfortable position and his attention diverted. In the balance, no activity was noted.

In an earlier paper, one of us (J.S.D.) described action currents in human muscle which, when palpated, was abnormally tender and resistant to pressure deformation. The muscles examined were spinal extensors and the subjects were at rest in the prone position with the head in the midline. The activity was not constant in degree; at times it did not appear for a considerable period after the electrodes were placed (1–45 min.), and at others it came in and dropped out without apparent cause. The subjects, except one, had no organic disease. Each, however, had a postural abnormality. Because this activity seemed to be a definite departure from normal, and because the afferent limbs of the reflex arc were not observed objectively and hence must be considered in general terms, the muscle activity seen in these studies was termed a "lesion reflex," and the areas called "lesion areas."

Since it had been noted in most lesion areas that activity could be induced by merely touching the electrodes, not a normal finding in our experience or in that of others[1] (4), it was apparent that the needle stimulus summed with a subliminal C. E. S. (6), to cause reflex action.

Wright (18) cites the work of Mackenzie who postulated that muscle contraction, persistent or evoked by palpation, might be an exaggerated

[1] Buchthal and Clemmeson, in commenting on the temporary effect of needle irritation state "Immediately after pricking with a needle electrode, there can appear in some muscle fibers a short (<1 min.) irritation with corresponding muscle discharge (pricking potentials). We have made the same observation. When the electrodes are in normal muscle, the skin about them may be moved to a considerable degree without initiating action potentials."

reflex response due to an area of "irritable focus" probably located in the posterior horn and created by an abnormal stream of impulses from certain pathological viscera or serous membranes. It seems probable that a pathological focus in structures other than viscera or serous membranes might create an area of "irritable focus" and thus might account for spontaneous or easily induced reflex activity.

Because of the importance of such activity, particularly in bio-economy and fatigue, further observations are reported.

METHODS

Dual channel, balanced (Offner Type 140) amplifiers drove a cathode ray oscilloscope, a loud speaker, or Westinghouse bifilar oscillographs for recording on bromide paper. In most of the experiments concentric needle electrodes (22 gauge) were used. Two needle electrodes and a needle and a skin pad electrode were used occasionally.

The subject was placed in the prone, sitting or standing position. When the subject was prone, the face was placed in a padded slot in the table; when sitting or standing the subject assumed a comfortable, "balanced" position except in a few experiments when records were made with the subject either standing on one foot or the other, or standing in a position of full forward flexion. Areas of lesion and normal control areas were selected by palpation and electrodes inserted. Electrodes and skin were treated with 70 per cent alcohol and the skin was anesthetized by intradermal injection of 1–2 cc. of 2 per cent procaine hydrochloride. The differential criteria between lesion and normal areas have been described elsewhere (5). In this series lesion areas have been classified arbitrarily as "major" and "minor." Major lesions are characterized by considerable abnormal firmness, rigidity and tenderness. In most instances the subject complained of having had discomfort in the area. Minor lesions are less rigid and tender and the subjects were usually unaware of the abnormality. Normal areas are soft, resilient and not tender.

When spontaneous activity was not present in a lesion area, stimuli were applied either halfway between the electrodes in the lesion and normal areas or equidistant from each. These stimuli consisted of slight movement of the electrodes, pin pricks or pin scratches, too light to cause mass contraction in the area,[2] cubes of ice with a surface area of approximately 2.5cm.^{2}, and pressures of 1000–1500 mm. of Hg delivered through a round cork which had a surface area of 175 mm.^{2} Forced inspiration and changes in position and weight bearing were also used.

Most experiments occupied approximately one hour. During this period constant observations of the lesion and normal areas were made with the oscilloscope and loud speaker. Photographic records were made at various intervals with the subject relaxed and in the quiet period between exhalation and inhalation (most of the muscles observed were accessory muscles of respiration.) We have found, as did Lindsley (12), that relaxation of normal muscle is not difficult to attain. Because voluntary contraction and involuntary tension show activity similar to reflex contraction, all records were taken with one electrode in a lesion area and another in a nearby normal control area. It was assumed that absence of activity in the control area was adequate to show an absence of either voluntary contraction or involuntary tension. While it is acknowledged that a trained subject might isolate voluntary activity to the lesion area, leaving the control quiet, these subjects were not trained, and care was taken to avoid letting them know what channel was feeding the speaker.

The areas examined included the spinal extensors, the glutei, the tibialis anterior and the extensor digitorum longus.

SUBJECTS

Thirty-two records were taken on 16 men and one woman, including students and instructors, all between the ages of 21 and 36. Fifteen presented some degree of postural

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[2] In a control experiment with both electrodes in normal areas, pin pricks sufficiently sharp to cause bleeding did not initiate activity. These were heavier than those used in other experiments.

abnormality. Six had been subjects in the previous series; all were free from gross disease and were carrying full work. Only one had been incapacitated from injury in the past five years. He suffered a fractured elbow one and one half years ago and made an uneventful recovery.

Although these subjects are ordinarily classified as "normal" or "healthy," those with major lesions have a history of discomfort, in varying degrees, in the lesion area. Likewise, these individuals report fatigue at physical effort. No measurements of this factor have been made. Two subjects were excellent physical specimens and provided good normal control observations. Except for a minor pollen sensitization in one, both have been unusually free from minor illness and have good capacity for work without fatigue.

Results

Spontaneous activity. This was considered to be present when one or more motor units were active in the absence of voluntary contraction or

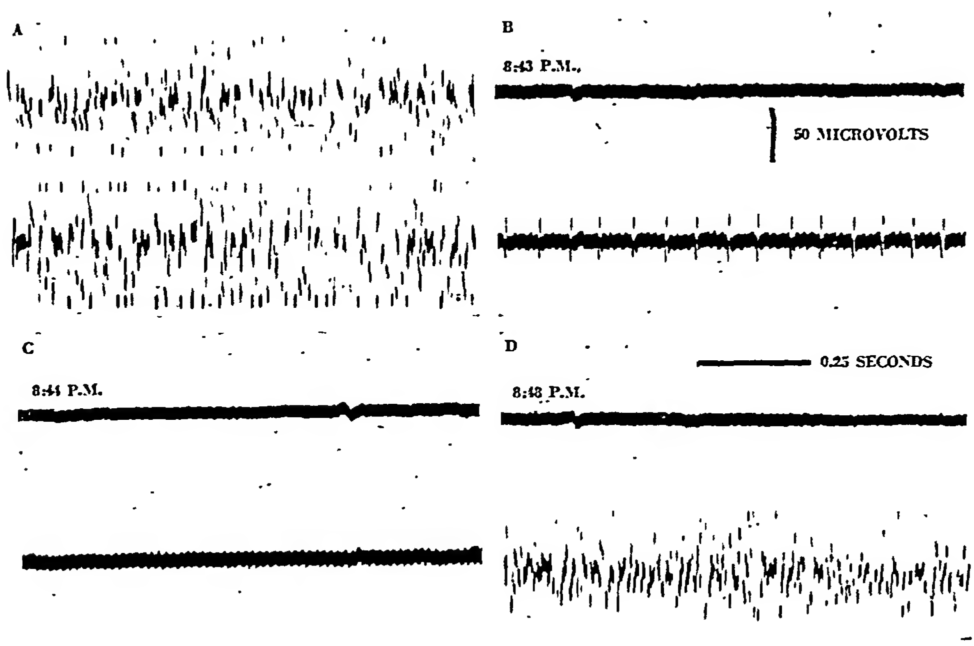

Fig. 1. Varying degrees of activity in lesion area. Upper tracing—concentric electrode in normal area about 3 cm. to left of sixth thoracic spine. Lower tracing—concentric electrode in lesion area about 3 cm. to right of sixth spinous process. (A) voluntary contraction to show that both electrodes were in muscle. (B) single unit firing in lesion area. (C) both areas quiet. (D) many units active spontaneously in lesion area.

involuntary tension after the flurry of activity induced by electrode insertion subsided. This activity was seen in twenty out of twenty-five observations of areas of major lesion. It waxed and waned in degree, varying from quiet periods to single unit activity to mass contraction (Fig. 1). No sharp pattern for the rise and fall of activity could be detected. Factors which usually increase the frequency and number of units firing, without affecting the nearby control area, are inhalation, painful stimuli, apprehension and fatigue from being in one position. Factors which usually decrease the fre-

quency and volume of activity are a deep breath taken slowly, a slight change of position and mental concentration. However, a given factor might cause a decrease in activity at one time and an increase at another. Two examples can be cited.

When a single unit is active in a lesion area in the thoracic extensors, the frequency of firing often increases with inspiration (Fig. 2). In two experiments attempts were made to get photographic records of this phenomenon. One subject became tense while receiving instructions and many additional units became active. In the other, while instructions were given, the active unit faded and was quiet for six minutes when other units came in spontaneously. In the latter experiment the quiet control area fed the speaker while the activity in the lesion area was switched to the oscilloscope,

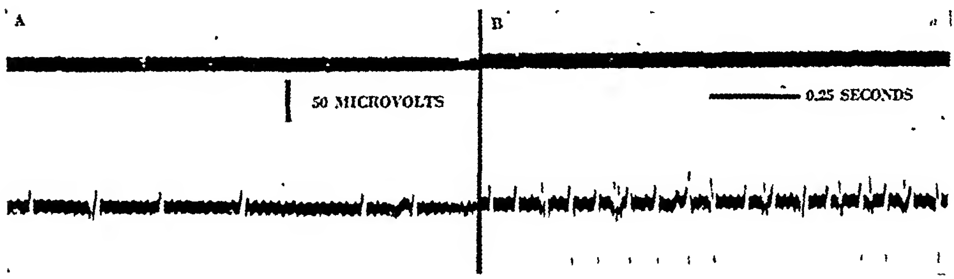

FIG. 2. Varying frequency of single unit during inhalation and rest periods of respiratory cycle. Upper tracing—concentric electrode in normal area about 3 cm. to right of fourth thoracic spine. Lower tracing—concentric electrode in lesion area 3 cm. to right of *sixth thoracic spine. (A) subject inhaling. (B) rest period between active exhalation and* inhalation.

out of the vision of the subject; he was unaware of the progress of the experiment.

Often there was rotation of spontaneous activity, different units coming in or dropping out without apparent cause. In one experiment (Fig. 3) a single unit fired during the rest period before inhalation. It persisted through inspiration and part of exhalation but faded during the ensuing rest period. Seven seconds after the beginning of the first, the next inhalation started. Units different from the original single one became active. Meanwhile, the control area remained quiet. Four minutes later the original single unit started to fire spontaneously.

Absence of spontaneous activity. In 5 records from areas of major lesion there was no spontaneous activity found in observations which extend over 65, 95, 82, 49 and 117 min.

In 4 experiments on 4 subjects there was no spontaneous activity in minor lesions. In two of these during other experiments, the muscle rigidity was more marked and spontaneous activity was shown.

Induced activity. We often noted that a slight movement of the skin around an electrode in the lesion area was followed by motor unit activity while the control area, similarly treated, remained quiet. This suggested a

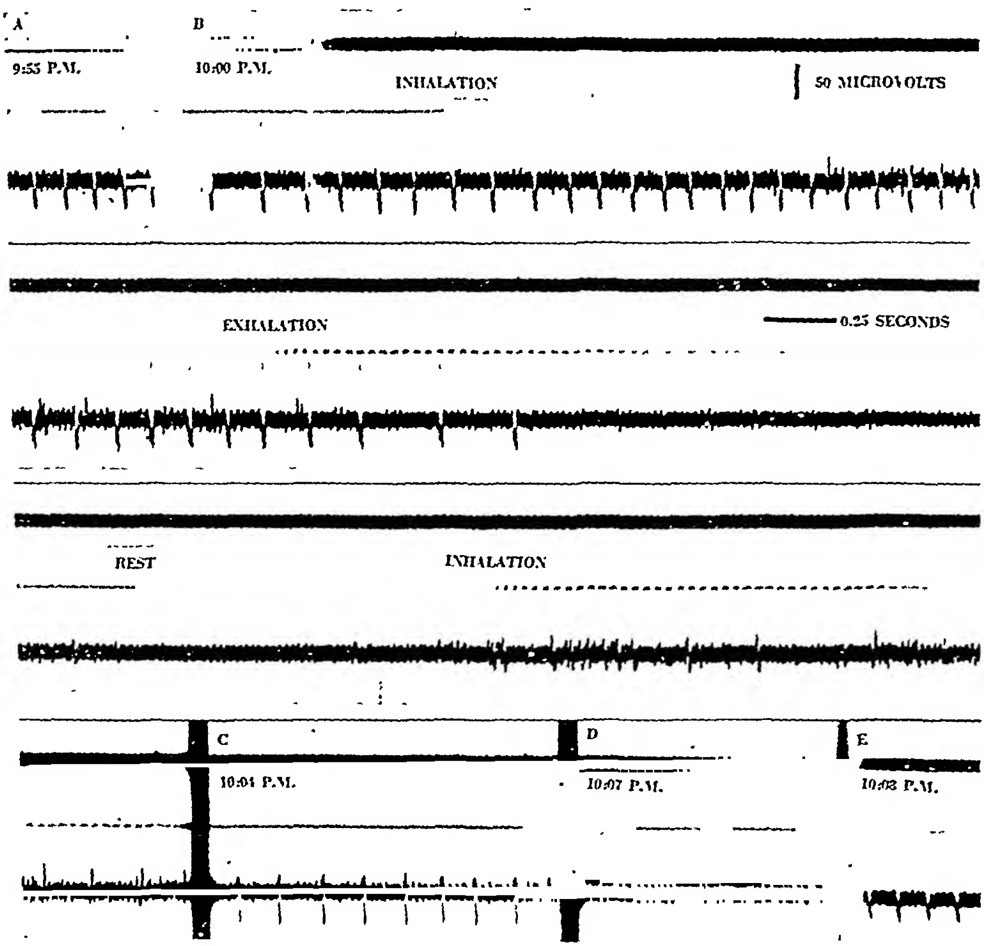

FIG. 3. The effect of respiration on reflex activity of the spinal extensors at the level of the fifth thoracic vertebra; concentric electrodes in normal (upper tracing) and lesion (lower tracing) areas. (A) single unit firing. (B) phases of respiratory cycle as marked. (C) spontaneous renewal of activity in lesion area. (D) extinction of unit shown in (C) after movement by subject. (E) spontaneous renewal of activity lesion area, apparently by the same unit shown in (A). Time marker (0.25 sec.) shown in (A) and (D) used in (B) to indicate change in phase of respiration.

means of differentiating, by means other than palpation, a normal area from a lesion area in which no spontaneous activity was occurring. Accordingly, when neither normal nor lesion areas showed activity after the electrodes were placed, the area between the electrodes (generally the skin over the spinous processes) was stimulated by pricking or scratching with a needle, by direct pressure or by the application of a cube of ice. Occasionally such stimulation evoked no activity but in general motor unit activity in the lesion area began soon after its application. Figure 4 shows a record of such activity induced by scratching the skin.

In the present series of experiments, stimuli as described above were applied fifty-five times. Activity in the lesion area was evoked forty times (72.7 per cent) and in control areas only five times (0.9 per cent). Activity thus induced in control areas was always transitory, while in the lesion areas it frequently persisted over relatively long periods (up to 10 min.).

In addition, two subjects who presented no obvious symptoms of neuromuscular lesions were used as controls. Out of ten applications of various stimuli, only once was there evidence of even minor activity.

Subjects sitting, standing erect, standing flexed, standing on one foot. It is interesting to note that in the spinal extensors in a few experiments the activity or lack of activity characteristic of the normal and lesion areas does not vary greatly when the subject is in a weight bearing position,

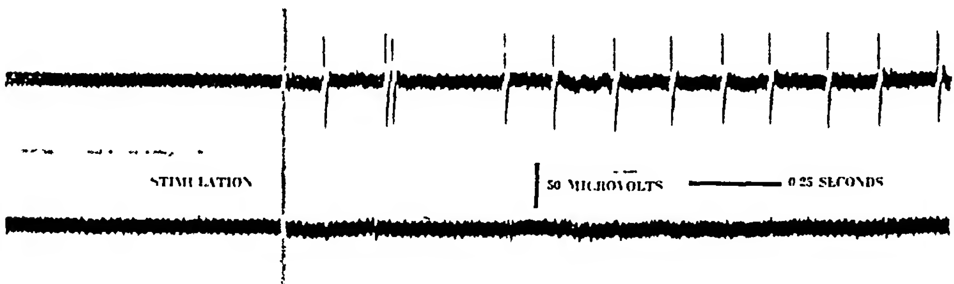

FIG. 4. Motor unit activity induced in a lesion area by scratching the skin between the electrodes. Upper tracing, lesion area; lower tracing, normal area. Time from application of the stimulus (indicated by cessation of time marker) to beginning of activity, 4.5 sec.

provided he is able to stand in an easy position. Further observations are being made and will be reported at a later date.

Activity in control area. There is no sharp line of demarcation between a lesion and a normal area, the former shading into the latter. At times, to get a nearby control it was necessary to put the electrode in the fringe of the lesion. If spontaneous or easily induced activity was seen, the needle was moved to a quiet area. If the activity was occasional and if the area was quiet while activity in the lesion area was being observed, it was considered a valid control. Likewise we sometimes erred in differentiating normal from lesion areas, probably due to the depth of tissue.

Non-rhythmic action potentials. Smith (16) reported irregular frequencies and stated "at threshold a unit may discharge in quite a random way without establishing any rhythm at all." We have seen this in several experiments. In one experiment a photographic record which ran 27 sec. showed one unit which fired twice and one which fired four times. This type of activity was observed throughout much of the experiment (77 min.).

From time to time the action potentials appearing on the photographic record present unusual patterns. In Fig. 5, (A) shows double spikes which have appeared in several records. The latency of these double spikes is

approximately 8.0 σ. In the same figure (B) and (C) show bursts of activity which in (C) occur with some regularity. These bursts are composed of large single discharges followed by a shower of lesser ones. In the same record can be seen a rhythmic single unit which continued to fire after the bursts had ceased spontaneously (D).

Activity in muscles with a common nerve supply. In most experiments it was difficult to identify accurately muscles under examination. In one, however, the tibialis anterior was the lesion area, the homolateral extensor

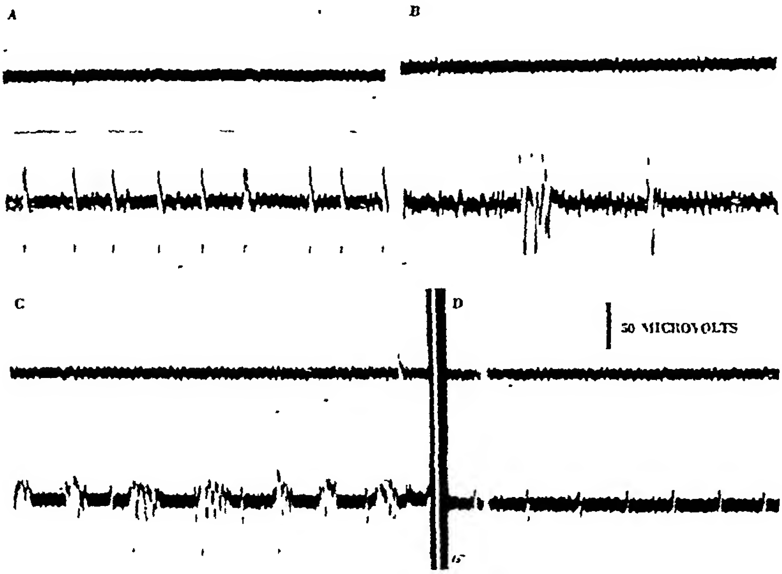

Fig. 5. Unusual types of action potentials. (A) double spikes; (B) irregular spikes. (C) small single spike combined with bursts. (D) same single unit firing alone 2 min. later.

digitorum longus being the control. There was a sharp difference in rigidity and tenderness; the latter was normal and the former quite firm and tender. There was alternating rhythmic and random activity in the lesion with the control quiet for 77 min. Obviously, although the deep peroneal nerve supplies both muscles, they receive motor fibers from different areas in the anterior horn.

The results in hand as regards spontaneous muscle activity, the recruitment and the frequency variation of such activity, as well as its evocation during quiet periods, confirm the observations which were reported in an earlier paper (5).

Discussion

Since the rhythmic action potentials of single motor unit activity were originally shown by stimulating single nerve fibers (1, 2), it has been established that such activity, when it occurs *in vivo*, is the result of reflex action.

The motor unit is the "final common path" (6, 14); its activity is the result of afferents reaching the spinal cord at a given level, hence it may be used as an indirect indicator to ascertain the level of any bombardment of afferent impulses.

Jacobson (10), Lindsley (12), Smith (15), Seyffarth (13), Hoefer and Putnam (7), and Buchthal and Clemmesen (4) have reported an absence of muscle action currents in the normal resting muscle. This is supported by our observations. Hence, we have concluded that the presence of spontaneous or induced activity in such muscles is abnormal. Since this activity occurs commonly in muscles which are rigid and in areas of postural abnormality, it strongly suggests that these are related and interdependent phenomena. Although the rigid muscles in the lesion areas resemble, in texture, muscles in voluntary contraction, the spontaneous or induced activity in the lesion area is usually less than that of voluntary contraction.

In a single clinical experiment on a case of suspected tetanus, Watkins (17) used the effect of an external stimulus on the action potentials of the affected muscles to confirm the diagnosis and to follow the process of recovery. The electromyograms showed that the sound of a whistle greatly increased the action potentials of the affected muscles, even though they were ostensibly relaxed. This indicated that the state of hyperirritability characteristic of tetanus existed, and that the central nervous system was involved.

The evocation of additional activity by a stimulus which, in the normal would be ineffective, is explained by Sherrington's[3] (14) concept of a motoneuron pool in which there is an enduring subliminal central excitatory state (C. E. S.) created by subthreshold stimuli.

Apparently, the phenomenon of spatial summation operated to cause massive blocks of motoneurons to fire. Although Watkins' study was made on tetanus, and our own on rigid muscle in postural abnormalities, our observations of action potentials resulting from a minor stimulus parallel his.

In this phase of the discussion the size or cause of the enduring C. E. S. is less important than the fact that such states have been observed in supposedly healthy individuals.

Steindler (16) regards vertebral joints as being capable of absorbing ordinary stresses and strains in the distributive mechanism of the ligamentous apparatus; he points out that static disalignments, certain anatomic types and anatomic abnormalities disrupt this mechanism with resultant symptomatology. This coincides with our observations that the rigid muscles occurring in postural abnormalities show electromyographic characteristics different from muscles which have normal texture.

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[3] The discussions of the enduring C. E. S. indicate that it lasts from ten to twenty σ. We have been unable to find data on the time it may persist with repeated stimuli which are subthreshold for either direct activity or summation. Our observations indicate that the subliminal C. E. S. may persist for indefinite periods, weeks at least. In one subject activity was induced in the lesion area in three experiments in an eight-week period.

However, the fluctuations in activity and particularly the change of threshold for various stimuli imply more fundamental changes than the merely uncomfortable orthopedic disturbances. In but one aspect, a study of the difference in metabolism between normal resting muscle without electrical activity and the spontaneously active or hyperirritable muscle should lead to knowledge which will provide a better understanding of the mechanism of fatigue.

Fluctuations in activity in the lesion area might result from changes in the environment of the afferent end organs, from extraneous factors affecting reflex activity in a given cord segment or, less probably, from changes in the motor unit. Further speculation at this time would be unfruitful, as direct studies of afferent activity have not been made and the limited knowledge of C. E. S. and C. I. S. precludes the possibility of drawing direct parallels.

The significance of the various types of arhythmic potentials which appear at times is very doubtful and it is hoped that more evidence can be accumulated. It is very interesting to note, however, that the double spikes shown in Fig. 5 are seemingly identical with those shown in a previous report (5). These spikes might be explained on the basis of: (i) double discharges of a single motor unit; (ii) other units firing almost synchronously; (iii) asynchronous discharge of fiber groups in a single unit.

Summary

1. Rigid muscles (lesion areas) associated with postural abnormalities commonly show spontaneous action potentials.

2. When such spontaneous activity is absent, it can be induced by suitable stimuli; this is a useful criterion for detecting lesion areas.

3. Induced reflex activity is probably dependent upon the presence of an enduring C. E. S. plus an accessory subliminal stimulus.

4. Various unusual types of action potentials occur; these are of doubtful origin.

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THE FUNCTION OF COMPONENTS OF THE RESPIRATORY COMPLEX[1]

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TRANSECTION of the brain stem has been widely used in the past as a method of delimiting the minimum respiratory neural complement necessary to support life. From such studies it appears that the caudal part of the bulb below the level of entrance of the eighth cranial nerves, the spinal motor pathways and certain reflex connections, constitute the essential parts of this mechanism (9). More recently the confines of the bulbar respiratory center have been more exactly mapped by localized electrical and chemical stimulation and by the recording of spontaneous action potentials in phase with respiration (4, 7, 8, 12, 24). These investigations confirm and add a measure of refinement to the localization established by gross transection.

However, Marckwald (19) and later Lumsden (18), Stella (29) and Pitts, Magoun and Ranson (26) described a more rostral division of the central respiratory mechanism subserving, along with vagal inhibitory afferents, the important function of contributing rhythmicity to respiration. This rather ill-defined rostral level was called by Lumsden the pneumotaxic center. The bulbar respiratory center, freed from inhibitory mechanisms within the upper brain stem and released from those stretch afferents within the vagus nerves which inhibit inspiration, passes into a state of maintained tonic activity resulting in inspiratory cramp (Marckwald) or apneusis (Lumsden).[3] Stella (30), especially, has strengthened the view that the inspiratory cramp results from unrestricted activity of the inspiratory center by showing that the magnitude of the cramp, *i.e.*, the depth of the maintained inspiration, is directly related to the carbon dioxide content of the inspired air.

Activities of individual inspiratory motor neurones have been studied extensively by a number of investigators in animals in which the entire neuraxis was intact (2, 6, 11). The behavior of these motor neurones under a variety of experimental circumstances is well known. It was felt that observation of the pattern and character of this neural discharge could be

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[2] Lucius N. Littauer Fellow of Neurophysiology.

[3] It is our impression that the term *pneumotaxic center* has given the false impression of a compact aggregate of neurons anatomically and functionally homogeneous, all of which must be eliminated to produce inspiratory cramp. More in line with experimental fact, is the view that there are a number of reflex connections at various levels within the upper and middle brain stem, the elimination of a sufficient proportion of which leads to inspiratory cramp. It is for this reason that we have returned to the less specific term, *brain stem inhibitory mechanisms*. Use of the term *apneusis* has unfortunately been construed as meaning the acceptance of Lumsden's terminology and views in their entirety. We therefore feel justified in returning to Marckwald's original descriptive term, *inspiratory cramp*.

used to assess, more accurately than observation of respiratory records alone, the effects of transection of the brain stem of the cat at various levels and of section of the vagus nerves. Transection methods are subject to criticism because of the associated shock and trauma and it is our belief that these factors can be evaluated better by a study of the neural discharge than of the respiratory records.

METHODS

The experimental methods employed for recording action potentials of phrenic neurones and respiratory volume of the cat are essentially those described by Pitts (23). The respiratory recording system was arranged so that the soda-lime absorption tube could be quickly shunted out of the system to test the animal's response to carbon dioxide. Transection of the brain stem was performed under ether anaesthesia. In the ensuing two hours or more, required in the preparation of single phrenic neurones, the greater part of the ether was eliminated from the body. Hence anaesthesia is not a complicating factor in these experiments.

Studies have been made in animals with the brain stem transected at either of two general levels. The more rostral of the two levels was the usual intercollicular section of Sherrington. The more caudal level passed through the acoustic tubercles dorsally and either the caudal pons or trapezoid body ventrally. In all instances the brain was removed at the end of the experiment and examined grossly for level and completeness of transection. Mortality was high in the animals transected at the caudal level despite prolonged artificial respiration. Of those animals which survived the low transection, some, on section of the vagus nerves showed slow prolonged rhythmic inspirations at a frequency of 2–4 per min. (19), sufficient to maintain life for a considerable period of time. The majority, however, showed true inspiratory cramp, *i.e.*, when the vagus nerves were cut, inspiration occurred at once and was maintained until the animal died in from four to eight minutes. Only results obtained on the latter type of preparation are considered here.[4]

Criteria of Normal Phrenic Activity

In a study of the pattern of activity of respiratory motor units, Gesell. Magee and Bricker (13) have pointed out that phrenic units characteristically show a slowly augmenting repetitive response during inspiration which ceases abruptly with the onset of expiration. Examples of this slowly augmenting and rapidly decreasing response may be seen in Fig. 1 to 5. Almost all phrenic units which we have observed, either with or without anaesthesia, show this type of response so long as respiration continues rhythmic and regular.

Depth of respiration has been shown by Adrian and Bronk (2), Bronk and Ferguson (6) and Gesell, Atkinson and Brown (11) to be regulated in two ways: (i), by the frequency of discharge of the several motor units and (ii), by the number of motor units active. These observations have been repeatedly confirmed in our experiments on phrenic motor neurones (Fig. 4 and 5). Together with observations of the pattern of activity, they constitute criteria of normality by which we may assess the effects of transection of the brain stem and section of the vagus nerves.

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[4] In a recent preliminary report Nicholson and Hong (20) have described a somewhat different respiratory response of the dog to combined section of the pons and vagi. A decision as to whether these results indicate a species difference between cat and dog or are dependent on other factors must await a complete report of their results.

Mechanisms Determining the Pattern of Inspiratory Activity

Eupneic respiration is ordinarily an active process only with respect to inspiration, expiration resulting from passive recoil of elastic chest structures. Observation of activity of phrenic motor units provides, therefore, an adequate picture of the neural processes of eupneic breathing. In Fig. 1, the top record shows the activity of two such phrenic motor units, distinguishable by differences in magnitude of spike potential. Both units show a slowly augmenting frequency of response during inspiration, which suffers sudden decrement with the onset of expiration. The records of Fig. 1 were obtained from an intercollicular decerebrate animal in which both

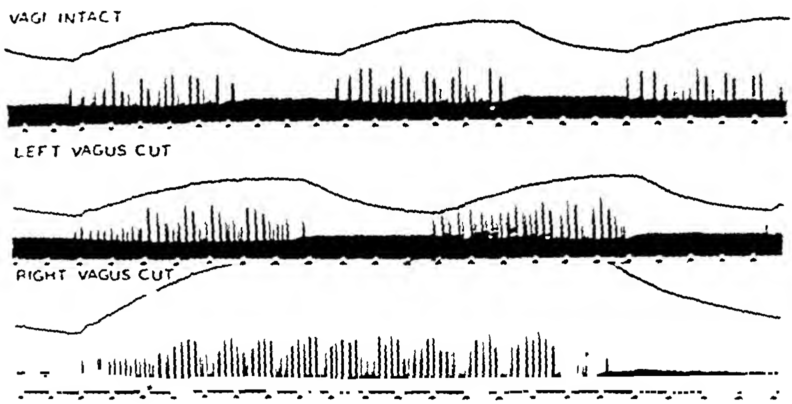

FIG. 1. The effect of section of the vagus nerves on the discharge of impulses by two phrenic neurones of a high decerebrate (intercollicular) cat. Upstroke of the respiratory record indicates inspiration. Time: $\frac{1}{5}$ sec.

the brain stem and vagus mechanisms which inhibit inspiration were intact. Section of first the left and then the right vagus was followed by the well known prolongation of inspiratory activity and slowing of the rate of respiration. The loss of those stretch afferents in the vagus which normally cut short inspiration (1, 15) is undoubtedly the major causal factor in the prolonged inspiratory activity seen in the lowermost record.[5] The general pattern of activity on the other hand, is not significantly altered. Inhibition of inspiration after bilateral vagotomy is eventually brought about by activity of those brain stem inhibitory mechanisms which remain intact in the intercollicular decerebrate animal.

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[5] In confirmation of the results of Larrabee and Knowlton (16) we have seen no evidence of activity of stretch afferents in the vagi which excite inspiration during normal eupneic respiration (14). The rate of augmentation of frequency of phrenic neurone discharge is essentially the same with vagi intact as with *vagi cut*. The peak frequency of response is, as a matter of fact, usually higher after vagal section, and recruitment of additional neurons often occurs. Both results are probably dependent in part upon higher carbon dioxide content of the blood due to less effective ventilation.

The low decerebrate animal, in which these mechanisms have been eliminated, breathes in an essentially normal manner so long as the vagi are intact (upper record of Fig. 2). The characteristic slowly augmenting and rapidly decreasing frequency of phrenic neurone discharge is evident. Section of the left vagus prolonged inspiratory activity and slowed the rate of respiration. Bilateral vagal section led to continuous repetitive discharge of this neurone, and to the recruitment of another, characterized by a spike potential of greater magnitude. The picture is one of an even more complete removal of inhibitory influences acting on the respiratory center than that seen in the lower record of Fig. 1.

A partial restoration of conditions essential to rhythmic respiration may be effected by intermittent stimulation of the vagus with low intensity, high frequency stimuli (26). A study of the behavior of phrenic neurones under these conditions reveals how very closely the activity pattern of this

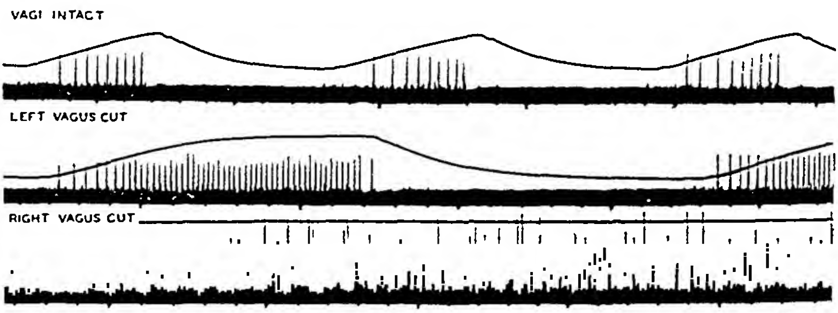

FIG. 2. The effect of section of the vagus nerves on the discharge of impulses by two phrenic neurones of a low decerebrate (low pontine) cat. Upstroke of the respiratory record indicates inspiration. Time: ⅕ sec.

artificially induced rhythmic respiration approximates the normal. Figure 3A is a continuation of Fig. 2 after bilateral vagus section. In Fig. 3B and C, repetitive central stimulation of one vagus, for the time indicated between the arrows, restored respiration to something approximating that of unilateral vagal section (Fig. 2, middle record). A slow augmentation of frequency of phrenic discharge and a relatively rapid decrement is evident. Higher intensities of vagal stimulation, though leading to more rapid inhibition, invariably showed evidence of brief excitation prior to inhibition (possibly the excitatory vagal afferents of Gesell and Moyer, 14). In general features, however, the respiratory pattern of record C approximates the normal quite well.

An additional feature of Fig. 3 is worthy of note. Inhibition resulting from a relatively short (about 0.8 sec.) burst of stimuli to the vagus, persists for some time (1.4 to 3.0 sec.) before activity recommences. Furthermore, the neurone of greater spike potential (Fig. 2, bottom record, and 3A and B, first part) is maintained inactive with brief repetitive central stimulation of the vagus at the intervals shown in Fig. 3B and C. It seems

possible that those central inhibitory changes which lead to cessation of inspiration during stimulation, persist in diminishing degree for an interval after stimulation ceases. The augmenting frequency of phrenic neurone discharge at the onset of the next inspiration might thus be a function, in part, of the progressive decay of this inhibition.

If one compares the pattern of inspiratory discharge determined by activity of the brain stem inhibitory system (Fig. 1, bottom record) with that determined by vagal afferents of an inhibitory nature (Fig. 2, top record, and 3C) the fundamental similarity is evident. In normal eupneic respiration the vagal inhibitory mechanisms are probably the more domi-

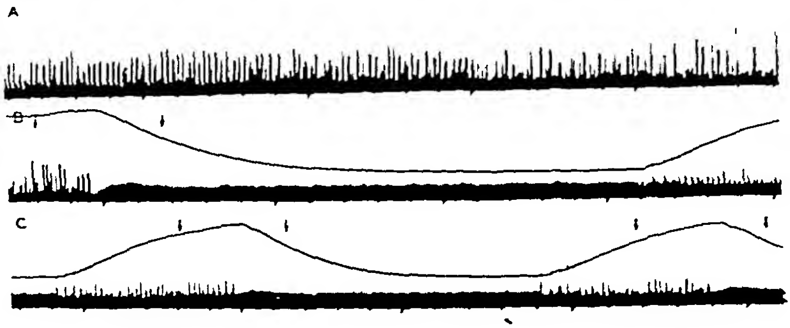

FIG. 3. The effect of stimulation of the central and of the right vagus nerve of a bilaterally vagotomized low decerebrate animal on the discharge of impulses by two phrenic neurones. Record A, a continuation of the lower record of Fig. 2; B and C, stimulation of the vagus during the interval between the pairs of arrows with shocks of near threshold intensity and a frequency of 100 per sec. Upstroke of the respiratory record indicates inspiration. Time: $\frac{1}{5}$ sec.

nant ones, since section of the vagi leads to a profound slowing of respiration (at least initially). It is possible, however, that there is some mutual reenforcement of the activities of the brain stem and vagal inhibitory systems.

MECHANISMS DETERMINING DISCHARGE FREQUENCY AND RECRUITMENT

The ultimate stimulus to the respiratory center is probably a chemical one. Carbon dioxide, within limits, excites the respiratory center to a degree proportional to its tension in the blood stream. Translated into terms of normal phrenic neurone discharge, with increase of chemical stimulus the frequency of discharge of individual neurones is increased, inactive neurones are recruited, activity of certain neurones begins earlier in the inspiratory cycle and in addition the rate of respiration is increased. The operation of these factors is evident in the records of Fig. 4 (A to D), taken from a small slip of the phrenic nerve of an intercollicular decerebrate animal. The soda-lime absorption tube was shunted from the system and a gradual

accumulation of carbon dioxide was effected by rebreathing. The increase in frequency of discharge is evident in the response of the neurone of greater spike potential; recruitment of another neurone of lesser spike potential is evident in records C and D. As a consequence of the operation of such mechanisms, inspiration increased in depth. Rate of respiration also increased significantly from A to D.

Repetition of rebreathing in the same preparation after bilateral vagal section yielded results shown in Fig. 4 (E to H). These records were taken at time intervals approximately equal to those of Fig. 4 (A to D). Respiratory rate was slowed markedly as a result of vagotomy and showed no increase during rebreathing. However, frequency of impulse discharge in-

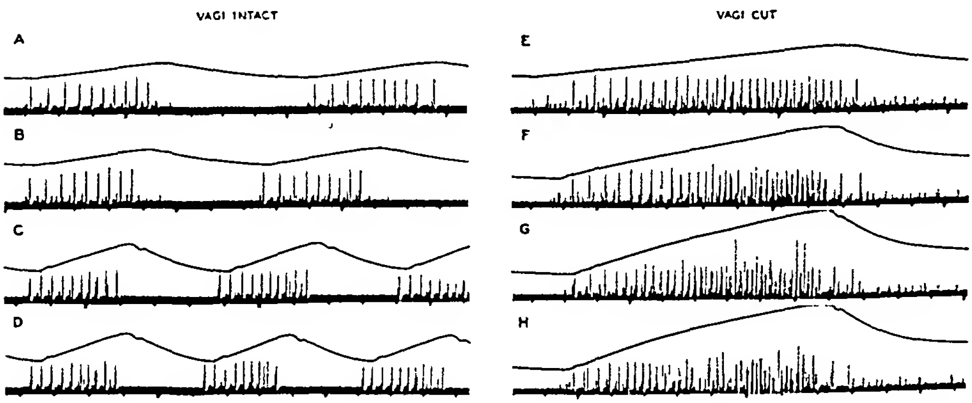

FIG. 4. A comparison of the effects of increasing tensions of carbon dioxide in the inspired air on the discharge of impulses by a few phrenic neurones before and after section of the vagus nerves (high decerebrate preparation). A to D and E to H, approximately equivalent increases in carbon dioxide before and after vagal section. Upstroke of the respiratory record indications inspiration. Time: ⅕ sec.

creased much as it did with vagi intact and recruitment of additional neurones is even more evident. As a consequence depth of inspiration increased during rebreathing while rate remained essentially constant. Similar results have been obtained in several additional experiments.

Scott (28) and Larrabee (17) observed that the increase in rate in response to carbon dioxide inhalation is largely dependent on the integrity of vagal reflexes. Schmidt (27), however, has observed instances of rate increase after vagotomy, and observation of results obtained on a series of animals in our student laboratory indicate that in some a substantial rate increase may occur on rebreathing after bilateral vagal section. However, the mechanisms responsible for increase in impulse frequency and recruitment (the chief factors in increasing depth of respiration) are certainly resident in the brain stem below the level of intercollicular decerebration and are independent of the vagus nerves.

Repetition of these experiments in low decerebrate animals, *i.e.*, with

brain stem inhibitory mechanisms excluded, yield results, an example of which is presented in Fig. 5. This animal breathed rather slowly and deeply as shown in record A. Rebreathing, however, increased the respiratory rate some 30 per cent from A to C (measured on longer strips of record). In addition, the neurone of greater spike potential showed an increase in frequency of discharge, and in record C, another neurone of lesser spike potential was recruited. The response of the low decerebrate animal is in all fundamental respects similar to that of the intercollicular decerebrate animal as long as the vagi are intact.

Section of the vagi of the low decerebrate animal resulted in maintained phrenic discharge as shown in Fig. 5 (D to F). Record D followed immedi-

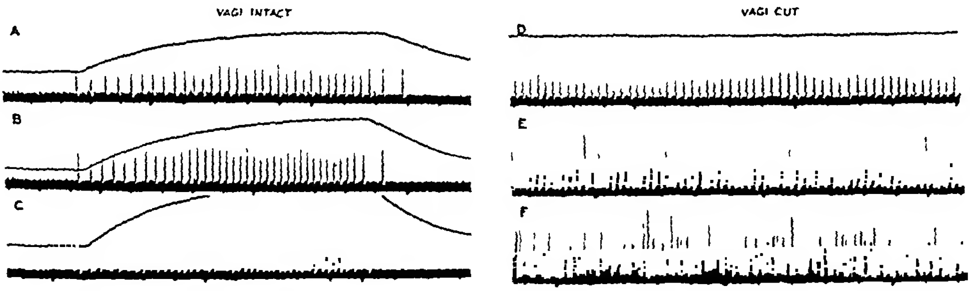

FIG. 5. A comparison of the effects of increasing tensions of carbon dioxide in the inspired air on the discharge of impulses by a few phrenic neurones before and after section of the vagus nerves (low decerebrate preparation). A to C, increasing carbon dioxide before vagal section; D to F, increasing carbon dioxide after vagal section. Upstroke of the respiratory record indicates inspiration. Time: $\frac{1}{5}$ sec.

ately upon vagal section, E and F, at intervals thereafter. Shortly after record F was taken the discharge became irregular and the animal died. Records D to F are comparable in a sense to the rebreathing records of Fig. 4 and 5 (A to C). Carbon dioxide certainly accumulated, and probably to a greater degree in this series than in any of the others. With carbon dioxide accumulation, an increase in frequency of phrenic neurone discharge occurs from D to F, and in addition there is a recruitment of new units as is evident in E and more marked in F.

It is apparent that mechanisms for increasing frequency of discharge and recruitment of inspiratory motor units may be experimentally isolated from those governing rhythmicity and rate of respiration. With elimination of those inhibitory systems imparting rhythmicity to respiration, there remain inherent in the respiratory center of the lower part of the bulb, mechanisms for recruitment and increase of frequency of the final motor units in response to an increase of carbon dioxide in the inspired air.

Discussion

The central respiratory mechanism may be divided for convenience into four systems: (i) the bulbar respiratory center-motor neurone system; (ii)

the vagal inhibitory system; (iii) the brain stem inhibitory system; and (iv) other excitatory and inhibitory systems.

1. The respiratory center-motor neurone system

Neurones of the inspiratory center making up the ventral part of the inferior reticular nucleus (21) are sensitive to changes in carbon dioxide tension of their fluid environment (8). Since the large residual and supplemental lung volumes effectively buffer the alveolar gas tension, and hence arterial blood, against any but slight tidal variations, the chemical stimulus acting upon these neurones may be considered essentially a constant one. In normal respiration, however, the response of these neurones to such a constant stimulus is phasic. Such phasic activity might result from processes inherent within the neurones of the respiratory center (3, 10, 31) or be imposed upon them by inhibitory mechanisms operating from without (18, 19, 29).

The present results are in accord with the latter thesis, for the combination of low transection of the brain stem and vagal section, eliminates such inhibitory mechanisms to a degree sufficient to convert phasic to maintained activity. Furthermore, they confirm Stella's contention (29) of the essential respiratory character of apneusis or inspiratory cramp in that they show, as did his results, that the magnitude of the cramp is related to the carbon dioxide content of the inspired air.

These studies, however, go further in showing that the inspiratory center-motor neurone system, in isolation, responds in a perfectly normal fashion to carbon dioxide stimulation. That is, the frequency of discharge and the numbers of final motor neurones responding, increase as the chemical stimulus is increased. But phasic interruption of this activity is lost and, as a consequence, the inspiratory pattern of slow augmentation and rapid decrement of discharge is not seen. However, this normal pattern may be restored by intermittent stimulation of the central end of one vagus. These results confirm our belief that inspiratory cramp is a specific response to elimination of inhibitory mechanisms, not a non-specific result of trauma of the section.

Studies of chemical activation of simple nervous structures, such as peripheral nerve and sympathetic ganglia, have shown that the individual units respond repetitively at frequencies proportional to the degree of disturbance of the chemical environment. Furthermore, additional units are recruited into activity as the degree of disturbance is increased (5). The similarity of the isolated respiratory center-motor neurone system to these simpler systems is sufficiently evident to require no further comment.

2. The vagal inhibitory system

Inspiration causes the lungs to expand, exciting stretch receptors of the parenchyma. These impulses, conducted centralward over the vagus nerves. lead to inhibition of phrenic nerve discharge (1, 16, 15). It is probable that these impulses, relayed through the nucleus solitarius, excite the dorsal reticular expiratory center (26). These expiratory neurones are functionally

interconnected with, and exert an inhibitory influence on the inspiratory center (23).

The magnitude of the inhibitory inflow over the vagi is proportional to the degree of lung stretch and hence to the depth of inspiration (1, 15). It is reasonable to assume that the ease with which the inspiratory center can be inhibited is inversely related to its level of excitation as revealed by the total respiratory effort. Thus with higher carbon dioxide tensions, inhibition is less easily effected and, as a consequence, deeper inspiration results. An increase in the frequency and number of active units brought about by the increased chemical stimulus produces an inspiration which attains a critical inhibitory depth sooner, *i.e.*, the slope of the inspiratory record is increased (Fig. 4, 5). The greater the level of excitation, the more rapid the recovery from inhibition and hence the shorter the expiratory pause.

Addition of the vagal inhibitory system to the respiratory center-motor neurone system imparts rhythmicity to respiration and provides the groundwork for increase of rate as well as depth of respiration.

3. The brain stem inhibitory system

The brain stem mechanism may be thought of as a subsidiary and possibly an adjuvant inhibitory system. It seems to operate in a manner parallel to the vagal inhibitory reflex system, but differs in that it lies wholly within the brain stem. Activity of the inspiratory center, relayed cephalically to higher levels, is returned caudally to the expiratory center along pathways in the ventro-lateral tegmentum of medulla and pons, leading to autoinhibition of inspiration (26). The slowing and deepening of respiration attendant on vagal section would seem to indicate that the brain stem inhibitory mechanism operates at a higher inspiratory level than the vagal reflex mechanism.

While we have no direct evident on the point, it would seem not unlikely that inhibition, which leads to the rapid decrement of frequency at the end of inspiration, persists through the expiratory pause and in diminishing degree into the start of the succeeding inspiration. The gradual augmentation of inspiratory discharge may be attributed in part to the gradual decay of inhibition.

4. Other excitatory and inhibitory systems

Afferents having origin in the various cranial nerves or in the sensory surface of the body and viscera impinge on either or both of the inspiratory and expiratory divisions of the respiratory center. Specific chemoreceptor afferents in the ninth and tenth cranial nerves play an important rôle in regulation of respiration under conditions of oxygen lack. Apparently the vagus afferents, described by Gesell and Moyer (14), which excite inspiration when the lungs are overdistended (15, 16), belong in this category also. In addition, cortical and hypothalamic connections modify respiration in important ways. Since our experiments have not dealt with these relationships, any discussion of their mode of action would be entirely speculative.

Summary

The central respiratory system may be functionally divided into four subsidiary systems: (i) the respiratory center-motor neurone system; (ii) the vagal inhibitory system; (iii) the brain stem inhibitory system; and (iv) other excitatory and inhibitory systems. The first three of these systems have been studied in isolation and in various combinations.

The respiratory center-motor neurone system regulates depth of inspiration by controlling motor unit impulse frequency and numbers of active units. The activity of this system in isolation is continuous and graded in degree in relation to carbon dioxide tension of the arterial blood.

The vagal inhibitory and brain stem inhibitory systems serve in a parallel manner to inhibit periodically the activities of the respiratory center-motor neurone system. Such periodic inhibition leads to rhythmic respiration and provides the groundwork for variation in rate.

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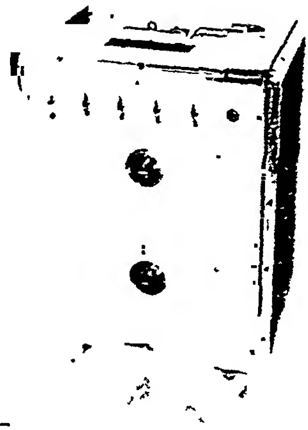

ABSENCE OF LOCAL SIGN IN VISCERAL RESPONSES TO PAIN

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A SYMPATHETIC response, manifested by vasoconstriction, piloerection, and sweating, may occur in response to painful, psychic, and other stimuli. In the case of painful stimulation, the question arises whether there is any tendency for localization of the response to the stimulated area. This paper deals with experiments designed to test for localization by measuring responses in man, both near and far removed from the origin of the stimulus.

This physiological information is of interest with reference to questions concerning disturbances of the autonomic nervous system as seen in patients with vasomotor changes and hyperesthesias, resulting from painful scars, causalgias, and other injuries. Livingston (8) reports ten cases of disability caused by pain in the extremities following slight trauma. Vasomotor phenomena, hyperesthesias, referred pain, and in some instances excessive pain were present. Stabins *et al.* (10) have shown that the efferent side of such a reflex need not necessarily be limited to the peripheral distribution in which pain originates.

A series of experiments on vasomotor control and sweat gland activity suggested a logical approach to the solution of the problem concerning localization of the sympathetic response. Many workers have shown conclusively with plethysmographic studies that a decrease in the volume of a part may follow painful, auditory and psychic stimuli (2, 7, 11). An increase in the sweat gland activity, measured in terms of skin resistance change, has been demonstrated with different types of stimulation (2, 3, 4).

By using methods for studying finger volume changes and skin resistance changes in the right and left hands of an individual, and by applying the painful stimulus to the dorsum of either hand, any tendency for localization of the response should be apparent. If any tendency for localization were present, the finger tip of the left hand should decrease in volume to a more pronounced extent than the corresponding finger tip of the right hand, when the left hand was stimulated and *vice versa.* Skin resistance changes were used as an additional check.

METHODS

Changes of finger volume were recorded optically, using a modification of the method described by Burton (2). The left and right index fingers (second and third phalanges) were placed in separate snugly fitting brass tubes, the junction between finger and plethysmograph being sealed with liquid adhesive compound. The plethysmographs were in turn connected by rubber tubing to a tambour, fitted with a rubber diaphragm and a small mirror chip. By air displacement, changes in finger volume caused a deflection of a light

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beam, which was reflected from a slit lamp to a recorder carrying sensitized paper. The tambour carrying the mirror chip was placed at a distance of one meter from the recorder. The two systems were equilibrated so that an equal change in each finger would cause an equal deflection of the light beams on the recorder aperture. Calibration of the plethysmographic response was done by causing a known volume of mercury, in a manometer, to displace an equal volume of air in the plethysmograph system, recording the resulting deflection of the light beam on the recorder. By this method the responses obtained could be expressed in volume change per cc. of finger mass. The latter was measured by water displacement. The possibility of leaks in the air conducting system was checked before each recording. Diminution of finger volume has been shown to occur on deep breathing (1). Assuming that the initiation for this reflex is common to both right and left index fingers, one might expect an identical response from both sides, as a result of a deep breath. If both systems were of the same sensitivity and free of leaks, a deep breath caused identical excursions of the light beams. Figure 1 is a typical curve illustrating such a response. This was found to be valid by checking with the volumetric method described above. Since the response resulting from a deep breath may be great at times, respirations were recorded optically during each experiment to obviate confusion with responses obtained following painful stimuli.

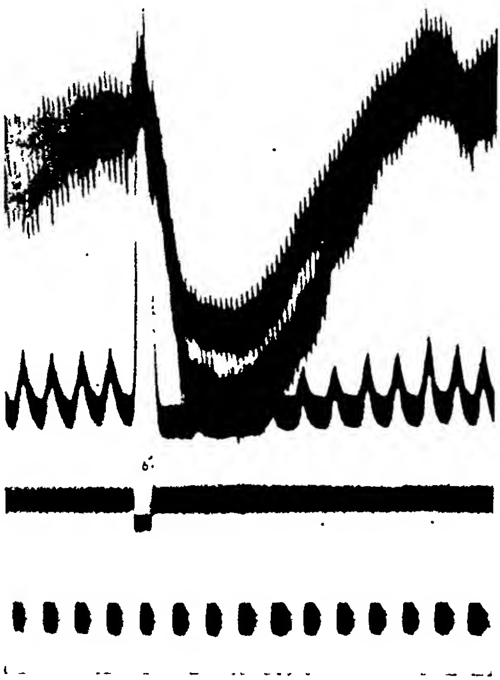

FIG. 1. This record shows the equal vasoconstriction obtained in both right and left index fingers following a deep breath. The uppermost curve is the right index finger, the second curve the left index finger. Respiratory excursions are recorded on the third line and the signal marker on the fourth line. The bottom line is time recorded in 5 sec. intervals.

Skin resistance changes were recorded* by the method of Geohegan *et al.* (5) on a two channel instrument (in place of the oscillograph), each channel consisting of a Wheatstone bridge, a direct-current amplifier and a magnetically operated stylus.† Tracings were made on stylograph paper (paper covered with a thin coating of wax) by the warm point of the stylus. Leads from the right and left ring fingers were connected to the proper channel of the instrument, the ground electrode being held under the tongue of the subject. Small zinc electrodes were applied over a thin coating of Cambridge electrode jelly. A uniform contact was maintained by fixation with scotch tape.

Painful stimuli were given according to a method of Hardy and Wolff (6). The light of a 1000-watt bulb was focused by two plano-convex lenses, and allowed to irradiate an area 3^2 cm. on the dorsum of either hand for 10 sec. By such a control of intensity and duration, a uniform painful stimulus was delivered in all of the experiments. Stimulations were done on both right and left hands during each experiment.

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* A word of thanks is given to Dr. Orlando J. Aidar whose assistance in this portion of the experiments made the technical procedures more easily executed.

† This machine was designed in this laboratory by W. A. Geohegan and built by the Rahm Instrument Company of New York.

Twenty-five experiments were carried out on twenty healthy first year medical students (male and female). Observations were usually done in the morning following breakfast. Each subject was blindfolded before the experiment began so that stimulations would not be anticipated. After the recording equipment had been applied, the subject was given a 15 minute rest period before starting the experiment. All experiments were done in a controlled temperature room at 26° C.

Results

In all the subjects tested there was no evidence suggesting a difference in the amplitude of the sympathetic responses in the right and left hand. Recordings of skin resistance changes were made in 12 subjects simultaneously with finger volume recordings, and positive responses were obtained in all of these. Marked decreases in finger volume were obtained in 18 of the 20 subjects. The remaining 2 subjects had similar responses but these were consistently smaller. The individual variations in the amplitude of the response was thought to be due to a difference in the individual as far as stability of the autonomic nervous system was concerned, and also to be dependent on the physiological status of the subject at the time of the recording. Experiments were repeated on a few of the subjects at a later date. Some subjects yielded greater responses to stimulation, while others had smaller responses, when compared to the previous studies.

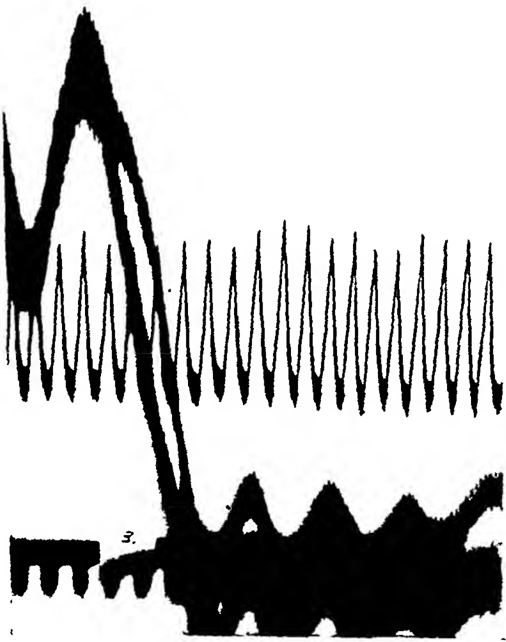

FIG. 2. Top curves are finger volume recordings of left and right index fingers with the right finger curve uppermost. Respiratory excursions are on the second line, with signal marker and time in 5 sec. intervals on the bottom line. The decrease in finger volume represented is approximately 0.01 cc. Note that the left and right fingers constricted equally to a painful stimulus of 10 sec. applied to the dorsum of the left hand.

Figure 2 is a typical curve of left and right index finger volume change following painful stimulation applied to the dorsum of the left hand. It is apparent that the volume decrease in the left index finger is identical with that of the right. This relationship held true in all responses obtained with an equal number of stimulations being done on the left and right hand of each subject. Stimulation of different sensory areas on the dorsum of the hand caused no differences in the responses. In Fig. 3 the light beams were

purposely superimposed to facilitate detection of any disparity in the responses of left and right. It is evident that the recording beams followed an identical path throughout.

In twelve of the subjects, while simultaneous recordings were being made of volume changes, skin resistance changes were also being measured. Figure 4 is a typical record of the changes of skin resistance following painful stimulation. The curves follow the same contour throughout. By calibrating the instrument against a known amount of resistance change, it was possible

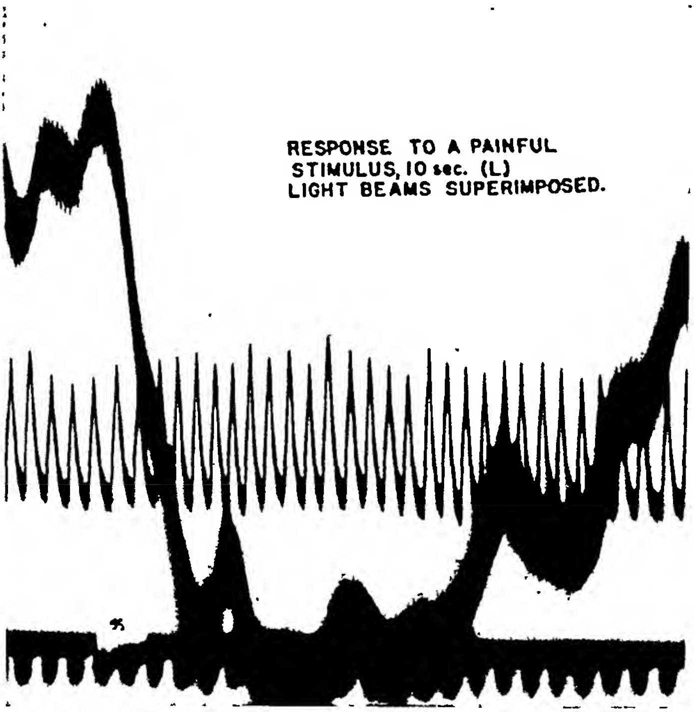

FIG. 3. Vasoconstriction in the right and left index fingers following a 10 sec. painful stimulus on the dorsum of the left hand. The light beams were superimposed in this record. It is evident that the two curves are identical since they appear as one. Respiration is recorded on the third line, signal marker on the fourth line with the time recorded in 5 sec. intervals on the bottom line.

to express the response as percentage change of the original resistance. Although the contours of the curves were similar, variations in the actual percentage change was found.

In three of the subjects, a greater response occurred always on the right side; the reverse was true in 3 other subjects. Of the remaining 6, the greater response occurred as often on the left as on the right, and in many instances the two responses were equal. Differences in the percentage changes between left and right were usually from 2–5 per cent. Differences as great as 10 per cent were observed on several stimulations. It should be emphasized here again that an equal number of stimulations was carried out on the right and left hands of each individual. The fact that the responses follow the same contour, and that the greater response (when obtained) could not be consistently demonstrated on the stimulated side, indicates little tendency for localization of this type of response.

Discussion

The outstanding factor concerning the autonomic nervous system is its tendency to go into action as a whole in the face of immediate danger or in severe environmental change. Such responses occur in severe physiological stresses of the individual. Other situations have been pointed out, in which one might expect "local sign" to appear. Although these experiments show such phenomena to be lacking for the particular groups studied, they do

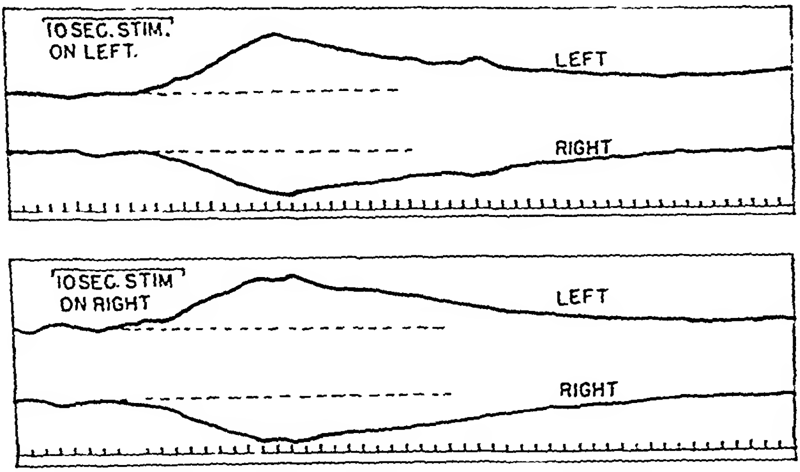

FIG. 4. Tracings of recordings of skin resistance changes in left and right ring fingers in response to a painful stimulus of 10 sec. applied first to the dorsum of the left hand and then to the dorsum of the right hand. The time is in one second intervals. Dotted lines represent projected basal resistances. The instrument was so arranged that a decrease in resistance in both channels would cause the recording styluses to diverge. It will be seen that the curves follow the same contour. The responses shown represent a 21 per cent change from a basal resistance of 19,000 Ω.

not necessarily obviate the occurrence of such in other instances. A prolonged stimulation, much less intense than was given here, might serve to initiate localization in a part. Other types of stimulation such as deep pain, repeated at frequent intervals, could result in prolonged vasoconstriction in a single part. In this series of experiments, a psychic stimulus (produced by telling the subject that he would receive an intolerable burn) caused as much vasoconstriction and skin resistance decrease, and in some instances more, than when an actual painful stimulus was applied. Neumann (9), in observing unmolested subjects over long periods of time with plethysmographic studies, has noted that although the spontaneous volume changes which occur in identical parts may follow identical paths for long periods, they may for a time drift apart. Occasionally the volume changes may be in the opposite direction in these subjects. Our observations have been directed to the response obtained after strong painful stimuli; spontaneous curves seen in these responses have followed one another closely.

A question has been raised concerning the decrease in skin resistance obtained following stimulation. Some believe that this response is purely cholinergic (4), *i.e.*, evidence of increased sweat gland activity alone. Others think that the response is due to blood vessel changes (adrenergic response), plus increased sweat gland activity. Carmichael (3) states that the skin resistance change can be elicited in the presence of one or the other, but is abolished in the absence of both vascular activity and increased sweat gland activity. Regardless of the interpretation placed upon skin resistance changes, it is an interesting fact that there was no significant difference in the responses obtained in the 2 sides of the body.

Summary

A series of experiments, designed to demonstrate localization of a sympathetic response in man following painful stimulation has been presented. Left and right index finger volume changes, obtained simultaneously, failed to show any difference in amplitude following a unilateral painful stimulus. Skin resistance changes recorded at the same time from the ring fingers bear a similar relationship. Although variations in percentage change of original skin resistance were observed at times, the greater change was not consistently present in the stimulated hand. These experiments indicate that there is no tendency for localization of the responses studied.

I wish to express my sincere thanks to Dr. J. C. Hinsey for his suggestions and assistance during the course of these experiments.

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POTENTIALS RECORDED FROM THE NERVE TRUNK AND THE DORSAL ROOT BY MICRO-ELECTRODES

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MICRO-ELECTRODES were used by Renshaw, Forbes and Morison (4) to record the potential changes that occurred in various regions of the brains of chickens, rabbits and cats under different experimental conditions. Similar electrodes were used by Therman, Forbes and Galambos (5) in a study of the response of the superior cervical ganglion of the cat to preganglionic stimulation. The results in the two groups of experiments suggested that micro-electrodes might provide the means by which definite information could be obtained concerning the electrical potential developed at the cell body of the neuron.

The anatomical arrangement obtaining in spinal ganglia, where the majority of the cell bodies are situated in a layer surrounding the fiber trunk, appeared to render these structures particularly suitable for a study of the potential developed by single cell bodies. The absence of typical synapses in these ganglia should also eliminate the complications of post-synaptic activity. Accordingly studies were begun on these ganglia using the glass micro-electrode described by Therman, Forbes and Galambos. The potential records were so complex and the interpretation so uncertain that a study of the potentials developed by the nerve trunk, as recorded by micro-electrodes, was made in an attempt to establish a basis for the interpretation of the records from ganglia.

METHODS

In all instances cats anesthetized with dial urethane (Ciba) were the experimental animals employed. The dorsal roots with their ganglia were exposed by laminectomy. The roots of the first and second sacral and the sixth and seventh lumbar segments are long and the ganglia readily accessible and therefore suitable for study of both ganglionic and fiber activity. These roots were used in all experiments. For recording the potentials of nerve trunks either the whole sciatic nerve or one of its bundles or a dorsal root, central to the ganglion, was the site for the electrode. In a few instances the sciatic nerve was excised, placed in a bath of Ringer's solution, and the potentials developed on stimulation recorded for comparison with those obtained with the nerve *in situ*.

Several arrangements of electrodes were employed for recording the action potential. A pair of silver-chlorided silver hooks spaced 1 cm. apart were used either with intact nerve on both electrodes or with the nerve killed at the distal electrode. When these electrodes were used, the stretch of nerve on them was freed from the rest of the tissue as much as possible. Also a single silver hook under the nerve, or a silver wire or disc on its free surface, was used in combination with a diffuse distant electrode consisting of a silver plate buried between skin and muscle on the side opposite to the location of the discrete electrode. Micro-electrodes were arranged to record against a distant diffuse lead or against a silver hook on the killed end of the nerve or another micro-electrode.

The micro-electrodes were of the glass type filled with Ringer's solution. They were placed as desired in the nerve or ganglion by means of micro-manipulators. The recording

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system was the cathode-ray oscillograph and associated amplifiers described by Therman, Forbes and Galambos.

Controlled activity in the nerve or ganglion was obtained by stimulation of the sciatic nerve or one of its branches peripheral to the site of the recording electrodes. The stimuli were condenser discharges controlled by a neon tube circuit and passed through a special transformer. These stimuli were applied once per second and were synchronized with the cathode-ray sweep by having the stimulator tripped by the cathode-ray sweep circuit.

Results

Nerve. The potentials developed by a stimulated nerve trunk when it is isolated and placed on the conventional paired electrodes have been

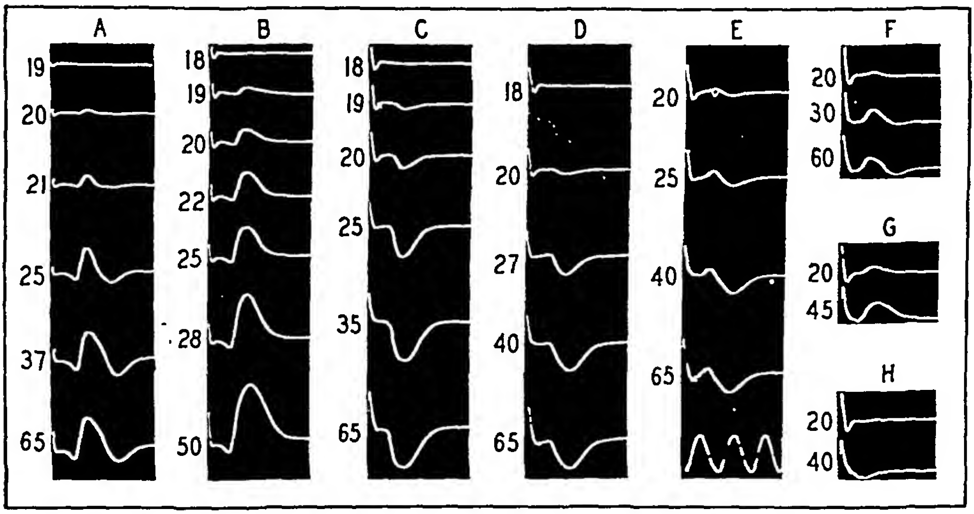

Fig. 1. Comparisons of the potentials recorded from a stimulated nerve trunk by a pair of large electrodes and by a 30μ micro-electrode.

The numeral to the left of each record indicates the strength of the stimulus. A, intact nerve on a pair of large electrodes spaced 1 cm. apart. B, nerve over distal electrode of the pair killed. C, 30μ micro-electrode substituted for proximal electrode of pair, 1 cm. from the border of the killed part of nerve. D, 30μ electrode 6 mm. from border of killed nerve. E, 30μ electrode 4 mm. from border of killed nerve. F, 30μ electrode 2 mm. from the border of killed nerve. G, 30μ electrode at junction of intact and killed nerve. H, 30μ electrode well within killed nerve. Time record in E is 2000 cycles/sec. and applies to all curves.

thoroughly studied, and the form of the potential record obtained under these conditions is well known. The potential changes in the stimulated nerve as recorded (i) by paired large electrodes and (ii) by micro-electrodes are shown in Fig. 1. When the intact nerve is placed on a pair of electrodes, spaced 1 cm. apart, the anticipated diphasic potential is recorded (Fig. 1-A). When the part of the nerve overlying the distal electrode has been killed the potential change is monophasic (Fig. 1-B). The records of Fig. 1-A and B are identical with those from completely isolated nerve except that there is a small initial positive deflection.

Figure 1-C shows the records obtained when a 30μ electrode is substituted for the proximal lead of the pair used in the previous experiments. The 30μ electrode was so connected that activity (negativity) in the nerve under it gave a downward deflection. The micro-electrode was first placed 1 cm. from the border of the killed stretch of nerve which rested on a large silver electrode. The records are identical in form with those obtained with paired electrodes but are somewhat reduced in amplitude.

Figure 1, D to H, demonstrates the change in form which the potential curve undergoes as the micro-electrode is brought nearer the killed part of the nerve. When the micro-electrode is a suitable distance away from the killed part of the nerve a typical monophasic record is obtained (Fig. 1, C and D). As the distance between the electrode and the killed nerve is decreased an initial positive wave is recorded. This is first seen at 4 mm. distance (Fig. 1-E) and increases as the negative phase decreases until it is the only deflection recorded (Fig. 1, F and G). When the micro-electrode is well within the killed stretch of nerve only the shock artefact is recorded (Fig. 1-H). The presence of a positive wave alone indicates that the nerve impulse dies out just before reaching the position of the electrode. The same change in the form of the potential record occurs when gross electrodes are similarly arranged on the nerve.

The potential curve recorded by the 30μ electrode (Fig. 1-C) grows as the strength of the stimulus is increased in the same way as that recorded by gross electrodes (Fig. 1-B). There is no sudden appearance of a spike which persists over a considerable range of intensities of stimulation as would be expected if the micro-electrode were recording from a single fiber or a very small group of fibers. All the characteristics of the curves are such as to suggest that the micro-electrode was recording the summed potentials from all of the fibers of the nerve trunk.

All micro-electrodes with tip diameters of 15μ or larger gave potential records similar to those recorded by gross electrodes. A 2 mm. silver disc on the nerve trunk, when the other electrode is distant and diffuse, records the potential changes as in Fig. 4, III-c and d. The triphasic curve is identical with that obtained with a 66μ electrode recording against a distant diffuse one (Fig. 4, III-g and h) or a 16μ electrode similarly arranged. This form of curve is typical of the combination of a discrete electrode on the active tissue and a distant diffuse one when the discrete electrode is on the nerve some distance from both the site of origin and of termination of the nerve impulse (1, 2).

Selectivity of micro-electrodes. To be of greatest service a micro-electrode should be sufficiently selective to record only the potential set up in the element immediately under its tip. The experiments of Renshaw, Forbes and Morison suggested that this was realized in certain parts of the central nervous system. The conditions in the nerve trunk when many fibers are activated synchronously are quite different from those in the central nervous system where activity may be very asynchronous. It is, therefore, necessary

to establish by experiment the extent of selectivity attained by micro-electrodes when placed in a nerve trunk where many fibers are activated synchronously.

An approximate estimate of the actual selectivity (attenuation of potentials arising at a distance) of micro-electrodes was obtained by placing the electrode in or near a small nerve bundle of the sciatic trunk and recording the potential change between it and a distant diffuse electrode. Data showing the attenuation of the voltage as the electrodes were moved away from the active bundle are summarized in Table 1. When an excised nerve placed in a bath of Ringer's solution was employed the attenuation of the potential was much greater with a micro-electrode of a given size than for the same electrode with the nerve *in situ*.

Table 1. Voltage recorded by micro-electrodes of different tip diameters at various distances from the active nerve fiber.

Distance of active electrode from bundle	Electrode diameter in μ	Relative voltage	Stimulus strength, dial reading
0.0 mm.	42	100	15
3.0	42	40	15
5.0	42	36	15
0.0	42	100	50
0.2*	42	63	50
3.0	42	47	50
6.0	42	42	50
0.0	16	100	20
1.0	16	10.7	20

* The electrode was in the connective tissue surrounding the bundle and may have been between 0.1 mm. and 0.3 mm. from the active fibers.

An appreciable part of the potential produced by the active bundle can obviously be recorded with the electrode some distance away. The larger the electrode tip the greater the potential recorded at a given distance. Even with the smaller electrode (diameter 16μ) the potential when the electrode was 1 mm. away was $\frac{1}{10}$ that recorded when the electrode was in the active bundle. This is far less attenuation than is required for discriminating single fiber activity. It is not surprising that we failed to record single fiber responses from a nerve trunk, in which many fibers are active, with micro-electrodes whose tip diameters are 15μ or larger. However, the marked attenuation of the voltage recorded by a 16μ electrode as compared to that recorded by a 42μ electrode suggests that smaller electrodes may be desirable.

The use of two micro-electrodes. Records obtained when two micro-electrodes were used are shown in Fig. 2. The use of two micro-electrodes

did not increase the selectivity. Compare the curves recorded between a micro-electrode and a diffuse one (Fig. 2-1, 2, 3) with those recorded between two micro-electrodes (Fig. 2-4, 5, 6, 8, 9, and 10). The two micro-electrodes attenuated the voltage and slightly increased the complexity of the curves. Stronger stimuli increased these complexities to the point where the records could not be interpreted (records not shown). The complexities arise from

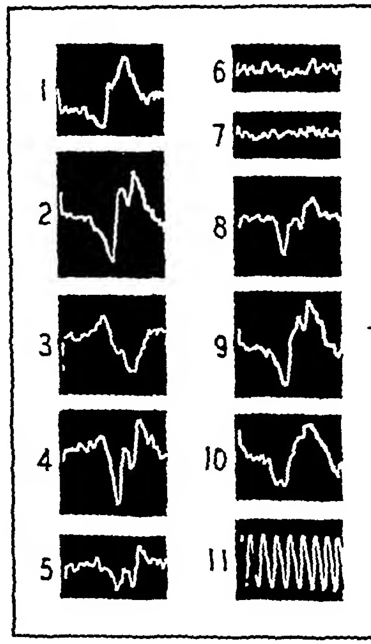

FIG. 2. Action potentials from the sciatic nerve as recorded by two micro-electrodes.
1, 2 and 3 between each of the micro-electrodes and a diffuse one; 4) recorded between a 42μ and a 32μ electrode, separated 1 cm. longitudinal to nerve trunk; 5) same electrodes as 4 separated 5 mm.; 6) same electrodes as 4 separated 2 mm.; 7) noise level, no stimulus; 8) electrodes separated 2 mm., stimulus increased to 3 times the value in 6; 9) electrodes perpendicular to longitudinal axis of nerve, separated 2 mm.; 10) electrodes separated 5 mm.; 11) time record 5000 cycles/sec.

the activity of different fibers under the two electrodes. Differences in conduction rate cause slight differences in the time of arrival of the nerve impulses at the two electrodes and sharp spikes appear in the record. These variables render the use of two micro-electrodes impractical in the nerve trunk even though the potentials from distant sources are cancelled.

The dorsal root ganglia. As a site for the study of cell body potentials the dorsal root ganglia seemed favorable. However, the variable contribution from nerve fibers rendered the potential records complex. The foregoing studies on the nerve trunk were made in an attempt to evaluate the fiber contribution. The conclusion was reached that electrodes greater than 15μ in diameter do not record single fiber responses. This conclusion usually applies to the ganglia also.

One type of record obtained from the ganglion when a micro-electrode is used with a diffuse one is shown in Fig. 3. When the electrode is on or just within the surface layer a triphasic wave is recorded (Fig. 3, A and B). As the electrode penetrates the ganglion more deeply the negative spike is re-

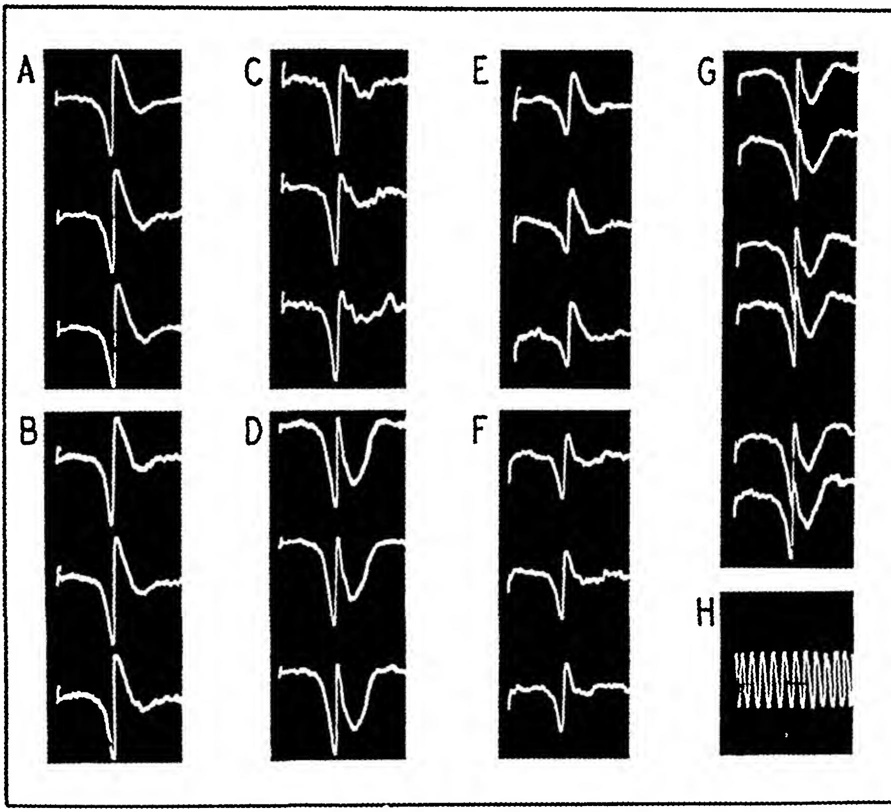

FIG. 3. Potentials from dorsal root ganglion recorded by a micro-electrode.
A. Potential recorded between a 20μ electrode and a distant diffuse one. Micro-electrode on surface of ganglion. Stimulus 20.
B. 20μ electrode just penetrating connective tissue covering of ganglion.
C. 20μ electrode about 0.7 mm. in ganglion.
D. Electrode deep in ganglion, ca. 1.5 mm.
E. Distant electrode replaced by a second micro-electrode on the connective tissue covering of ganglion. Electrodes separated about 1 mm. Original micro-electrode just penetrating ganglion.
F and G. Two different positions of original micro-electrode deeper in the ganglion than in E.
H. Time record, 4000 cycles/sec.

duced and a prominent later positive potential is recorded (Fig. 3, C and D). When the diffuse electrode is replaced by a second micro-electrode situated on the connective tissue layer outside the ganglion, records of similar form are obtained (Fig. 3, E, F, and G).

A reasonable interpretation of these records is that the "initial phases" (consisting of the initial positive deflection and the succeeding negative deflection as far as its crest) represent activity in the nerve fibers. The duration of the negative wave is slightly increased when the micro-electrode is on or just within the surface layer of the ganglion. The descending portion of the negative wave then represents summed potentials contributed in part

by nerve fibers and in part by cell bodies. When the electrode penetrates the ganglion more deeply its tip passes beyond many of the cell bodies and lies among the axon branches to these cell bodies. The late positive wave recorded from the depths of the ganglion probably represents the difference of potential existing between the cell bodies and their axonal connections. The part of the cell body attached to the axon branch must be activated first by the nerve impulses. The radial arrangement of the cell bodies makes it possible that part of the positive wave represents the difference of potential existing between two parts of the cell body as the impulse spreads over it. When the electrode is centrally located inside the spherical layer of cell bodies (near the collected axon branches) the positive wave should theoretically be greatest. This was the probable location of the electrode tip when large positive potentials were found experimentally.

The interpretation that the "initial phases" of the curves are produced by nerve fibers is supported by the reasonable value of the conduction rate (ca. 90 m/sec.) calculated on this assumption. The duration of the late positive phase (1 msec.) supports the interpretation that it is contributed by cell bodies. This value is comparable to that found by Renshaw (3) for the duration of activity in motoneurons of the spinal cord. The smooth contour of the curves suggests that the records represent the summed potentials from many units. The late positive wave from the ganglion is homologous with the terminal positive phase from the nerve trunk, but greater in amplitude and duration.

The interpretations regarding the sources of the recorded potentials are further supported by the observation that with a 2 mm. disc on the surface of the ganglion there is recorded a small later negative wave (Fig. 4, I-a). The same late negative wave is present when a 25μ electrode replaces the disc (Fig. 4, I-b). A large positive wave, out of which negative spikes rise, follows the "initial phase" when the micro-electrode is pushed into the surface layer of the ganglion (Fig. 4, I-c). When the stimulus is increased in strength the late negative spikes increase in size (Fig. 4, I-d). Our interpretation is that the positive wave represents the difference of potential existing between many cell bodies and their axon branches, and the negative spikes represent the development of negativity in a few cell bodies near which the tip of the electrode rests. The increase in size of the negative spike with a stronger stimulus indicates that more nearby cell bodies are activated.

When the 25μ electrode penetrates more deeply into the ganglion the positive wave persists but the negative spikes disappear (Fig. 4, I-e and f). Now the electrode has passed beyond the layer of cell bodies and its tip is surrounded only by axon branches. The positive wave means that these axon branches and proximal ends of the cell bodies are recovering as the cell body is invaded by the wave of depolarization.

Replacement of the diffuse electrode by a 25μ electrode in the same location attenuates the potential record. Strong stimuli elicit small negative spikes during the positive phase (Fig. 4, II-a and b). When the distant 25μ

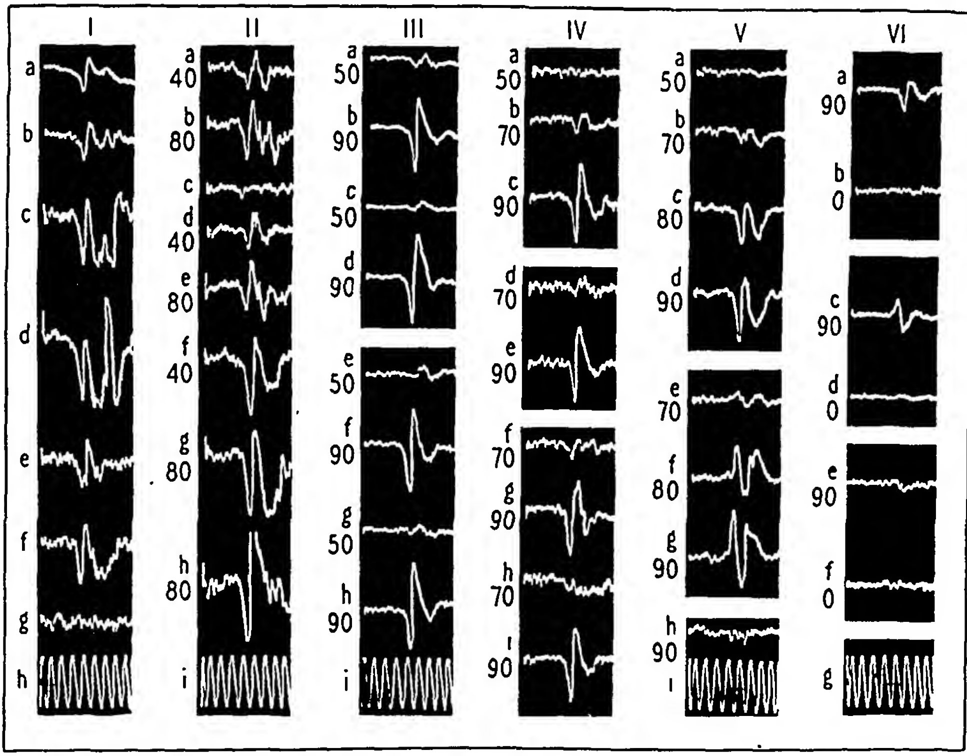

FIG. 4. Potentials from ganglia and dorsal roots.

I. a) 2 mm. silver disc on ganglion, other lead diffuse; negativity at disc deflection upward. Stimulus 40.
b) 25μ electrode on surface of ganglion, other conditions as in (a).
c) 25μ electrode penetrating upper surface of the ganglion, other conditions as in (a).
d) Stimulus increased to 80, otherwise as (c).
e) Micro-electrode pushed deeper in the ganglion, stimulus 40.
f) Stimulus increased to 80, otherwise as (e).
g) Noise level, no stimulus.
h) Time record, 2000 cycles/sec.

II. a) 25μ electrodes replace diffuse lead, 25μ electrode in ganglion same position as in (I-g).
b) Stimulus increased to 80, otherwise as (a).
c) Noise level, no stimulus.
d) Two 25μ electrodes, peripherally placed one penetrating ganglion; centrally placed one on surface of ganglion; negativity at peripiheral electrode deflection up. Stimulus 40.
e) Stimulus 80, otherwise as (d).
f) Centrally placed electrode now moved to a position medial to other micro-electrode. Penetrating electrode, lateral, and a diffuse one. Stimulus 40.
g) Stimulus 80, otherwise as (f).
h) Record between the two micro-electrodes. Stimulus 80.
i) Time record, 2000 cycles/sec.

III. a) 2 mm. silver disc on surface of ganglion against diffuse electrode. Negativity at disc upward deflection. Stimulus 50.
b) Stimulus 9 ... as (a).
c) Silver di ... rsal root central to ganglion. Stimulus 50.

electrode is moved to a position on the ganglionic surface 1 mm. central to the penetrating electrode the recorded curve is of the same general form (Fig. 4, II-d and e). Accidental displacement of the penetrating electrode has probably brought the tip nearer a few cell bodies so that now small negative spikes appear in the later part of the record. With the penetrating 25μ electrode in the same position and a diffuse lead, the positive wave is larger and shows traces of the negative spikes (Fig. 4, II-f and g). The record between the deep micro-electrode and another on the ganglionic surface medial to it shows a large negative wave followed by a small positive wave out of which small negative spikes rise. The form of all the potential curves presented in this group can readily be explained on the basis of the interpretation regarding the origin of the potentials set forth.

This interpretation also explains the form of the curve recorded from the ganglion by a 2 mm. disc (Fig. 4, III-a and b), and from the dorsal root (Fig. 4, III-c and d). The same explanation applies to the records obtained with a 66μ electrode on the ganglion (Fig. 4, III-e and f) and on the root

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d) Stimulus 90, otherwise as (c).
e) 66μ electrode on surface of ganglion against diffuse lead. Stimulus 50.
f) Stimulus 90, otherwise as (e).
g) 66μ electrode placed on dorsal root central to ganglion. Stimulus 50.
h) Stimulus 90, otherwise as (g).
i) Time record, 2000 cycles/sec.

IV. a) 20μ electrode on surface of ganglion. Stimulus 50.
b) Stimulus 70, otherwise as (a).
c) Stimulus 90, otherwise as (a).
d) 30μ electrode placed on dorsal root. Stimulus 70.
e) Stimulus 90, otherwise as (d).
f) 20μ electrode on surface of ganglion. Stimulus 70.
g) Stimulus 90, otherwise as (f).
h) 20μ electrode placed on dorsal root. Stimulus 70.
i) Stimulus 90, otherwise as (h).
Time record from III-i.

V. Two micro-electrodes penetrating ganglion, separated 1 mm. longitudinally. Negativity at 30μ electrode deflection up, at 20μ electrode deflection down.
a) Record between 30μ electrode and diffuse one, stimulus 50.
b) Stimulus 70, otherwise as (a).
c) Stimulus 80, otherwise as (a).
d) Stimulus 90, otherwise as (a).
e) Record between 20μ electrode and diffuse one, stimulus 70.
f) Stimulus 80, otherwise as (e).
g) Stimulus 90, otherwise as (e).
h) Record between 20 and 30μ electrodes, stimulus 90.
i) Time record, 2000 cycles/sec.

VI. 20μ and 30μ electrodes in dorsal root.
a) 30μ electrode and diffuse one, stimulus 90.
b) Noise level, no stimulus.
c) 20μ electrode and diffuse one, stimulus 90.
d) Noise level, no stimulus.
e) Record between 20μ and 30μ electrodes, stimulus 90.
f) Noise level, no stimulus.
g) Time record, 2000 cycles/sec.

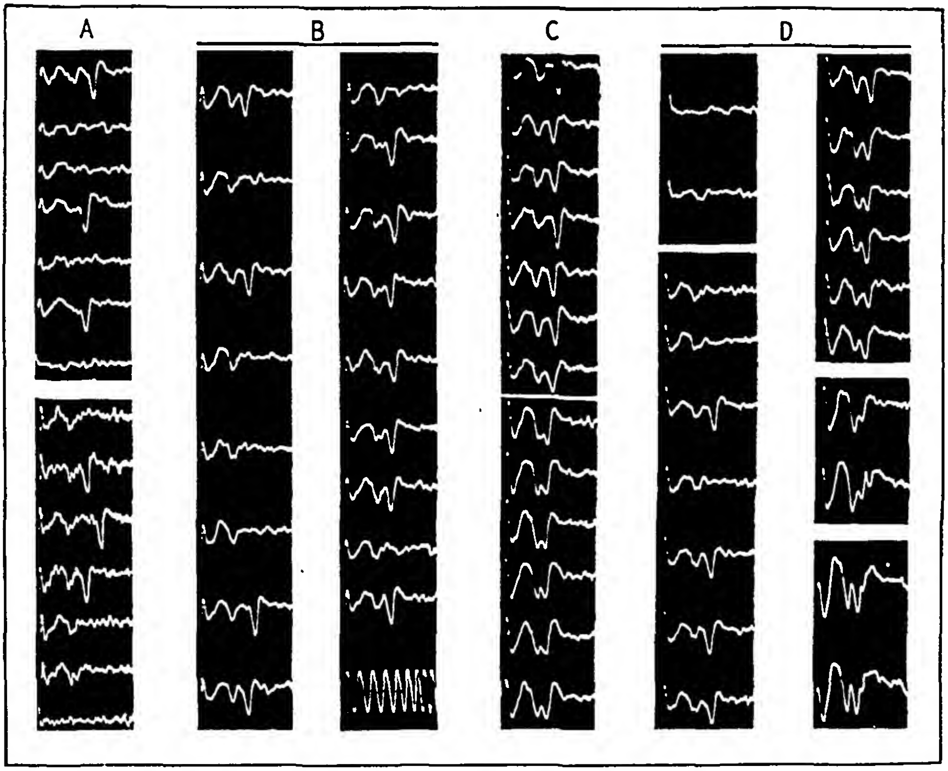

FIG. 5. Potentials recorded from single units of the dorsal root ganglion by micro-electrodes.

A. Two 20μ electrodes. One electrode just penetrating the surface layer of the ganglion. The second outside connective tissue covering of the ganglion. Negativity of penetrating electrode gives downward deflection. Upper portion, stimulus 12.5. Lower portion, as upper, but high frequency filter not used.

B. Stimulus 14; filter used throughout, otherwise as A.

C. Upper portion, stimulus 18. Lower portion, stimulus 20.

D. Selected portions of record obtained when the stimulus was increased from below threshold to maximal. Portions selected show characteristic changes in the potential record. Electrodes as in A. Time record 2000 cycles/sec.

(Fig. 4, III-g and h). With a 30μ electrode on the ganglion (Fig. 4, IV-a, b and c) and on the root (Fig. 4, IV-d and e) and also with a 20μ electrode similarly placed (Fig. 4, IV-f to i) the same form of curve is recorded with a slight attenuation of the potentials. The small late negative wave of the records is contributed by the cell bodies. It is small and smooth because the electrode is relatively far away from individual cell bodies not all of which become active at the same time.

Two micro-electrodes separated 1 mm. in an antero-posterior direction were pushed deeply into the ganglion. The curves recorded between the 30μ electrode and a diffuse one are shown in Fig. 4, V-a to d; those recorded between a 20μ electrode and a diffuse one in Fig. 4, V-e to f, and the curve

recorded between the two micro-electrodes in Fig. 4, V-h. A late positive wave characteristic of deep penetration is recorded by both electrodes. Between the two electrodes essentially no potential is recorded, which indicates that the potentials arose from sources equi-distant from both electrodes and were cancelled between them. The same pair of electrodes when placed on the dorsal root recorded potentials as seen in Fig. 4, VI-a to f. The records from both ganglion and root are of the form expected on the basis of our interpretation regarding potential origin.

In a few cases potentials that we interpret as the response of single units were obtained (Fig. 5). The negative spike (deflection downward) appeared with threshold stimuli (Fig. 5-A) and was still present with the same amplitude with maximal stimulation (Fig. 5-D, later portion of the record). The spike voltage is about 50μV. When the stimuli were near threshold the latency varied between 2 and 2.6 msec. (Fig. 5, A). This latency variation is characteristic of the single unit response when subjected to threshold stimulation. With stronger stimuli (Fig. 5, C, lower portion) the latency became constant at 1.7 msec. These stimuli also elicited another earlier spike of about the same voltage, and having a latency of about 1.4 msec. Since the spikes are negative they represent activity in cell bodies at the electrode tip. The presence of the two spikes indicates the location of the electrode tip near two closely approximated cells. That the selectivity of a 20μ electrode is adequate to record from one or two individual cell bodies is probable because the diameter of these cell bodies may be as much as 100μ. Therefore, when a 20μ electrode rested on two of them lying side by side it would be well isolated from most of the others.

On the basis of the conduction rate usually observed in these nerve fibers the nerve impulse should have reached the electrode in 1.1 msec. At constant latency of response the additional delays observed were for the early spike 0.15 msec. and for the later spike 0.4 msec. The early spike began 1.25 msec. and the later one 1.5 msec. following the shock artefact. The observed delay may be explained by a uniform but slow rate of conduction in the two fibers with a marked difference in rate for the two. The calculated rates are for the early spike 80 m/sec. and for the late one 66 m/sec. However, with stimuli as weak as those just necessary for constant latency of response in these units, we have never observed a conduction rate as slow as 80 m/sec. from the nerve trunk. A more probable explanation is that the rate of conduction was nearly the same in the two fibers as well as in the cell bodies, but that the rate of propagation is slower in the cell body than in the nerve fiber. The difference in the delay might then be due to differences in the distance from the point of entry of the impulse into the cell body to the point of contact with the electrode.

According to the interpretation of the origin of the ganglionic potentials that we have made, the spike represents negativity at the point of contact between cell body and electrode as the wave of depolarization invades the cell body. The same invasion of the cell bodies by the wave of depolarization

gives rise to the positive wave when the electrode is deeper in the ganglion, near the axon branches (Fig. 3).

Summary

Micro-electrodes have been used to record potentials from nerve trunks and from dorsal root ganglia.

1. The form of the potentials recorded from the nerve trunk by micro-electrodes is the same as that recorded by gross electrodes similarly arranged (Fig. 1).

2. The records are easier to interpret when one micro-electrode is used in combination with a distant diffuse one.

3. Micro-electrodes whose tip-diameters are greater than 15μ are not sufficiently selective to record single fiber responses from the nerve trunk when large numbers of the fibers are activated synchronously.

4. Small electrodes (16μ) attenuate potentials arising at a distance far more than do large electrodes (42μ).

5. The ganglionic potential curve is very complex when recorded by large micro-electrodes within the ganglion (Fig. 3 and 4). It is composed of waves representing activity of the nerve fibers contained in the ganglion and others representing activity of the cell bodies.

6. The various phases of the complex wave have been assigned to the various structures, and the alterations in the form of the curve with depth of penetration of the electrode explained.

7. In a few instances single cell-body potentials have been recorded from the ganglia (Fig. 5). This cell-body spike has a greater latency than can be explained by the conduction time in the nerve fiber and this delay probably represents a slowing of the depolarization wave as it invades the cell body.

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MEDIATION OF DESCENDING LONG SPINAL REFLEX ACTIVITY*

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THE EXPERIMENTS to be described represent an attempt to determine (i) the functional structure of that portion of the spinal mechanism utilized by long spinal reflexes, and (ii) the kinetics of central activity involved in long spinal reflexes.

Cats were used throughout the present experiments. The animals were made spinal by transection accomplished through the dorsal atlanto-occipital membrane. Artificial respiration was instituted and the ether anesthesia discontinued. To provide the afferent limb of the long spinal reflex, the brachial plexus was approached from the dorsum, crushed distally and equipped with stimulating electrodes. An afferent volley, resulting from a single stimulus delivered through the electrodes on the brachial plexus reaches the spinal cord by the lower cervical and first thoracic dorsal roots. This generally accepted statement has been confirmed by observing the cord potentials evoked at various levels by a volley of this kind. Figure 1, constructed after the manner of Hughes and Gasser (1, Fig. 1), represents plots of (i) the intramedullary spike potential (dots) and (ii) the negative intermediary potential (circles) at appropriate levels. The greatest influx of activity occurs in the lower three cervical segments. The skew deviation in the distribution of the intramedullary spike potential reflects the heavy contribution made by the ascending collaterals which form the fasciculus cuneatus (cf. also 9). The "tail" directed caudally undoubtedly represents conduction in the shorter descending collaterals. Van Gehuchten has stated (11) that in the rabbit the descending collaterals of the first thoracic segment extend as far as the eighth thoracic segment.

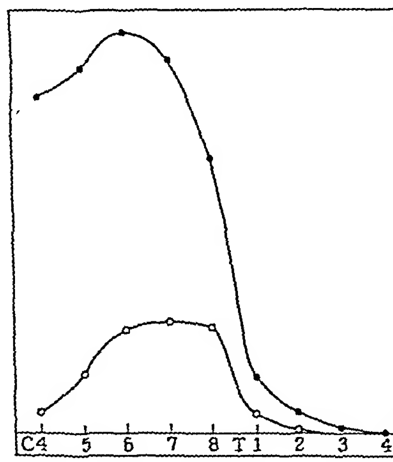

FIG. 1. Magnitude of intermedullary spike potentials (dots) and corresponding negative intermediary potentials (circles) evoked by a brachial plexus shock and plotted at various levels of the cervical enlargement.

Activity evoked in the cervical enlargement by a single brachial plexus volley has, in addition to spatial and intensity characteristics, a duration factor. The duration of the negative intermediary potential in this situation

* A preliminary account of some of the present results was presented at the meeting in Boston of the American Physiological Society (5).

is estimated by Therman (9) to be *ca.* 5 msec. It is known from analogous situations that internuncial activity, consequent upon dorsal root volleys, persists somewhat longer; in fact, no definitive statement relative to the duration of internuncial pool activity can be offered at the present state of knowledge, for various nuclei, subsequent to activation by an afferent volley, become silent at different times and in a roughly asymptotic manner.

In general it may be stated that vast pools of interneurons within the cervical enlargement become active for a period in excess of 5 msec. as the result of a single brachial plexus volley. It is the impulse output from these

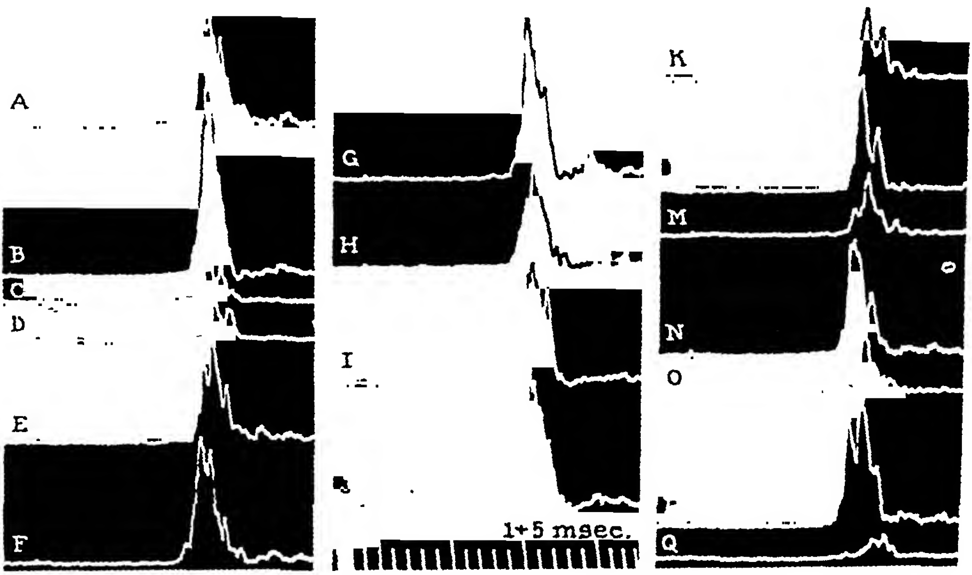

FIG. 2. Discharge of L7 motoneurons in response to an afferent volley in the brachial plexus. The records (all similar) were obtained with the use of ventral root leads and illustrate the variability of motor response.

active pools, directed caudally through the propriospinal system, that forms the input to the region under observation—the lumbar enlargement. Once below the caudal limit of active descending primary afferent collaterals, the responses to be considered pertain wholly to the intrinsic spinal system and to the motoneurons influenced by activity within that system. Thus in addition to the specific problem of the organization of long spinal reflex pathways, some of the present experiments have more general interest, inasmuch as they reveal features in the activity of pools of neurons when brought into play not by a synchronous nerve, root or tract volley, but by the diffuse, dispersed discharges projected from such other pools of neurons.

Records of the activity of the lumbar enlargement have been obtained, as in previous experiments (2, 4), by the use of microelectrodes placed at will within the cord, and by leads placed on selected ventral roots. Local

reflexes, occupying arcs of two neurons, have been employed to test the average excitability of selected motoneuron pools.

Long spinal reflex discharge. Figure 2 shows a series of long spinal reflex responses recorded in succession at 10 sec. intervals. In each case the afferent volley was provided by a standard brachial plexus shock; the efferent motor volley was recorded from the seventh lumbar ventral root (L7V.R.). One outstanding feature of the long spinal reflex discharge is its great variability, although variability in discharge is not unknown in even the simplest of spinal reflex arcs. Despite the intensity variation, however, the latency of the discharge is maintained relatively constant from one observation to another. In the 17 observations of Fig. 2 latency varies between 9.5 and 11 msec. A simple picture of the relationship between latency and intensity of the long spinal reflex discharges may be gained from the action potentials superimposed by the drawing in Fig. 3. Further examples to illustrate latency of the long spinal reflex discharge may be found in Fig. 4, 12, 13.

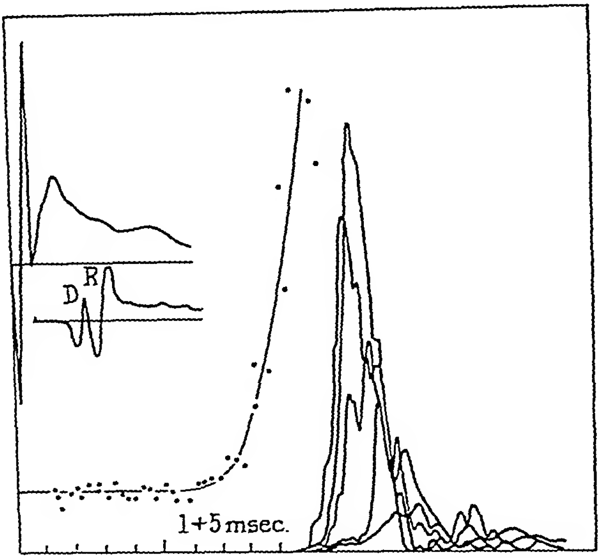

FIG. 3. Factors contributing to latency of long spinal reflex discharges. Top left: Cord potential evoked in the cervical enlargement by a brachial plexus volley. Next in order: Tract spike potentials recorded by microelectrode in the ventral column of the L7 segment in response to a ventral column shock delivered at the level from which the cord potential above is recorded. D, directly conducted tract spike potential. R, relayed tract spike potential (2). The tract spike potentials are drawn so that the shock artefact coincides in time with the onset of the negative intermediary potential above. Next in order: Graph showing onset of facilitation among L7 motoneurons as tested by a segmental two-neuron-arc discharge. Lower right: The discharge of L7 motoneurons. Five responses, including the extremes, are superimposed by drawing to illustrate variation in latency and intensity of the reflex discharge.

It may be noted that these experiments illustrate the extreme values for latency that have been encountered. The reflex latency is thus not only quite constant on successive observations on a single preparation; it is only slightly less constant from preparation to preparation. A further point of interest is the degree of synchrony with which the lumbar or sacral motoneurons discharge in response to a single brachial plexus shock. The discharge rarely outlasts 10 msec. and frequently is nearly complete in less than 5 msec., this in spite of the fact that internuncial impulses are contributed to the motoneurons and may be demonstrated as having a

facilitatory action there for a period in excess of 30 msec. (Fig. 15, 16). In summary then, the long spinal reflex discharge may be almost completely absent (Fig. 11), or it may approximate or even exceed (Fig. 12, 13) in size and synchrony the discharge obtained through short spinal reflex pathways. The latter fact is the more remarkable since as many as twenty spinal segments intervene between the afferent and efferent limbs of the long spinal reflex.

The course of events during the latent period for long spinal reflex discharge. The minimum reflex time for the long spinal reflex discharge approximates 9 msec. Figure 3 illustrates some of the factors to be considered in accounting for the observed reflex time. Assuming a single synaptic relay in the cervical enlargement between primary afferent fibers and long propriospinal neurons, and direct activation of some long propriospinal neurons by primary afferent impulses, the first impulses to reach the L7 segment would do so at *ca.* 2.5 msec. after the causal brachial plexus shock. This value is determined in the following manner. The cord potential of the cervical region evoked by a brachial plexus volley is recorded. This potential is drawn as the first record in Fig. 3. The onset of the negative intermediary potential signals the first internuncial activity resulting from the primary afferent volley. Stimulating electrodes are placed in the ventral column at the level previously used to record the cervical cord potential. A microelectrode is placed in the ventral column at the L7 segment and the tract spike potentials evoked by the ventral column shock are recorded as detailed in an earlier paper (2). For Fig. 3 the tract spike potentials of the direct (D) and relay (R) neurons are drawn in such a way that the stimulus artefact of the tract volley coincides in time with the onset of the negative intermediary potential of the cervical cord potential record above. The beginning of negativity at the microelectrode inserted into the ventral column measures the time at which, after the brachial plexus shock, the first propriospinal impulses might be expected to enter the L7 segment.

The value of 2.5 msec. must be taken as representing an order of magnitude, not a definitive value. In the first place the time at which the negative intermediary potential begins almost certainly marks the onset of internuncial activity in the dorsal horn. The synaptic delay indicated by this potential ranks among the shortest known. The largest propriospinal fibers characteristized by the highest conduction velocities might reasonably be supposed to convey the first impulses to the lumbar cord. These fibers belong to the ventrolateral columns (10) and undoubtedly have their cells of origin in the ventral horn. The synaptic systems of the ventral horn which have been adequately studied (primary afferent collaterals to motoneurons [6]; ventral column collaterals to short propriospinal neurons of the ventrolateral columns, and to motoneurons [2]) are characterized by somewhat longer delays in the "resting" cord. It is thus not unlikely that the longer delays should be allowed for the primary afferent-long propriospinal synaptic system. Secondly it is obvious that stimulation of the ventral columns activates descending systems other than purely propriospinal (2), with the implication that the ventral column record reveals predominantly vestibulospinal or reticulospinal activity. The force of the latter objection is mitigated by the fact that essentially similar records have been obtained in chronic spinal cats from whose cords extrinsic descending mechanism was eliminated by degeneration (unpublished observations). With due regard to these considerations, a value of 2.5 to 3.0 msec. is a fair and just approximation for the time of arrival of impulses in the L7 segment, given a single relay at the cervical level.

Facilitation of the lumbar motoneurons begins 6 to 7 msec. after the brachial plexus shock. The graph of Fig. 3 shows the early part of the facilitation curve of lumbar motoneurons, obtained by plotting the amplitude of a segmental two-neuron-arc discharge as a function of the interval by which the segmental test shock follows the conditioning brachial plexus shock. Between the delivery of impulses into the motor nucleus, as measured by facilitation therein, and the discharge of impulses from that nucleus there is, under the conditions imposed by long spinal reflex activation, a nuclear

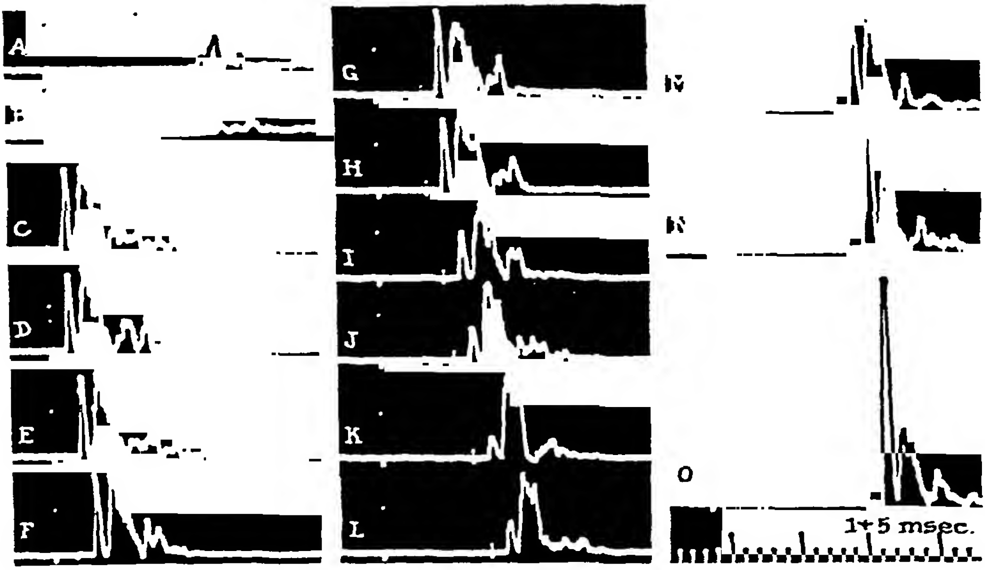

FIG. 4. Direct inhibitory action of propriospinal (internuncial) impulses on motoneurons as indicated by two-neuron-arc test reflex. A and B, response of L7 motoneurons to long spinal reflex activation. C, response of L7 motoneurons to segmental reflex activation. D-O, the segmental reflex tests the excitability of certain motoneurons during the latent period of the long spinal reflex. Note inhibition of the two-neuron-arc discharge, increasing progressively until the shock interval in K is attained; thereafter recovery and facilitation.

delay* of 2 to 3 msec., due allowance of course being made for ventral root conduction. It is clear from Fig. 3 that nuclear delay at the motor nuclei does not account for the total time between the hypothetical onset of tract activity in the L7 segment and the discharge of impulses from the motor nuclei. There remains an interval of 4 to 5 msec. preceding the onset of motoneuron facilitation for which some accounting must be made.

Direct inhibition. On many occasions when a segmental two-neuron-arc reflex discharge is utilized to measure the excitability of lumbar motoneurons following a brachial plexus shock, the first excitability change to occur is

* Nuclear delay is defined as an observed discrepancy in time between the arrival of impulses in a nucleus and the discharge of impulses from that nucleus under specified conditions of excitation.

not facilitation at 6 to 7 msec. after the shock, as in Fig. 3, 5 B, 5 C, but inhibition occurring with a latency of 2.5 to 3 msec. Figure 4 presents the results of such an experiment. Observations A and B of Fig. 4 illustrate the response of L7 motoneurons to single brachial plexus shocks, while 4C illustrates the response to a single L7 dorsal root (D.R.) shock. In Fig. 4 (D to O) the segmental reflex is introduced at increasing intervals after the brachial plexus shock, in order that the two-neuron-arc discharge may form a test of the excitability of certain motoneurons. In records D, E, F, and G no change occurs other than variation. From G to K, however, inhibition of the two-neuron-arc discharge occurs, increasing progressively, after which (L to O) inhibition is supplanted by facilitation. Graphs, further illustrating

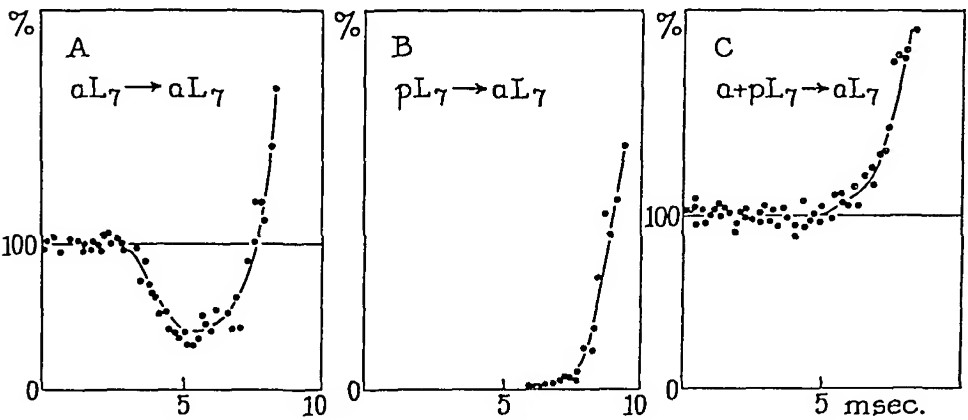

FIG. 5. "Masking" of inhibitory action. The effect of long spinal reflex activity on *motoneurons of the anterior half of the L7 segment (aL7) is tested in A by the two-neuron-*arc reflex evoked by a volley in the anterior half of the L7 dorsal root; in B, by the reflex evoked by a volley in the posterior half of the L7D.R.; and in C by the reflex evoked by stimulation of the whole dorsal root. The inhibition revealed by curve A is concealed in curve C. Further description in text.

the time course of inhibition in other experiments, are presented in Fig. 5A, 15, 16.

It is noteworthy that inhibition begins at the time predicted for the arrival of the first long propriospinal impulses within the L7 segment. A demonstrable excitability change in the motor nucleus at this time interval is powerful evidence that the propriospinal impulses do in fact arrive at the predicted time. The present example of inhibitory action is entirely analogous to that previously found to occur in short spinal reflex systems (3), with the important exception that the inhibition in this case is clearly attributable to the action of interneurons.

The masking of inhibitory activity. It is apparent from a comparison of Fig. 3 and 4 that all two-neuron-arc reflex discharges do not behave in a similar manner when brought into conjunction with long spinal reflex activity. Facilitation has always been observed as shown in Fig. 3, 5, 15, 16, but the earlier inhibition period is not infrequently absent. It might be

supposed that this fact indicates merely that some motoneurons receive inhibitory action coincident with the arrival of propriospinal impulses, whereas others do not. Some experiments have shown, however, that the fact may not be fully explained in this simple fashion. The results described in connection with Fig. 5, for instance, illustrate how an inhibitory action may be masked, presumably by virtue of some interaction within the testing system.

For each of the curves of Fig. 5 a standard brachial plexus volley provides the conditioning activity, and in each case the tested motoneurons lie within the pool contributing axons to the anterior half of the L7V.R. For curve A the test shock is applied to the anterior half of the L7D.R. and results in a two-neuron-arc reflex discharge, the amplitude of which is plotted in curve A as a function of the interval between the conditioning and testing shocks. In curve A inhibition begins *ca.* 3 msec. after the brachial plexus shock, and reaches a maximum between the 5th and 6th msec., after which facilitation supervenes.

Curve 5B is constructed in a manner similar to that of 5A. The test shock, however, is applied to the posterior half of the L7 D.R. There is no two-neuron-arc discharge in the control test responses, but it does appear by virtue of facilitation after a shock interval of *ca.* 6 msec. Since the two-neuron arc is subliminally active in the control responses, this test cannot indicate the presence or absence of an inhibitory period like that observed in 5A.

If now the whole L7D.R. be stimulated, a two-neuron-arc reflex response results, which is somewhat greater than that resulting from stimulation of the anterior half of the L7D.R. alone, due to convergence at the motoneurons of the primary afferent impulses of the two halves of the dorsal root. Curve 5C shows the effect of the long spinal reflex impulses as tested by the two-neuron arc evoked by stimulation of the whole L7D.R. No demonstrable excitability change occurs antecedent to the onset of facilitation in the 6th msec. Therefore, an interaction has taken place between the anterior and the posterior reflexes employed severally as tests in 5A and B respectively, on the occasion of their simultaneous combination. As a result, the inhibitory action of the long spinal reflex is either counteracted or masked.

Two interpretations of these observations seem worthy of consideration, although others undoubtedly could be developed. The choice between the interpretations depends upon whether one is to assume that the motoneurons responding after a single synaptic delay to stimulation of the anterior half of the L7D.R. alone do so when the whole dorsal root is stimulated, or conversely do not do so. Either assumption is reasonable. If motoneurons contributing to the test discharge employed in 5A also contribute to the test discharge of 5C, then it would seem that the convergence of anterior and posterior L7 impulses at the motoneurons, while not recruiting many more motoneurons into the test discharge (test response C is not much greater than test response A) has so fortified the synaptic drive within the firing zone that the inhibitory action of the long spinal reflex impulses is completely

overcome. Alternatively the posterior L7 dorsal root volley may be regarded as recruiting motoneurons heavily from the subliminal fringe of the anterior L7 reflex, the motoneurons so recruited not belonging to the fraction of

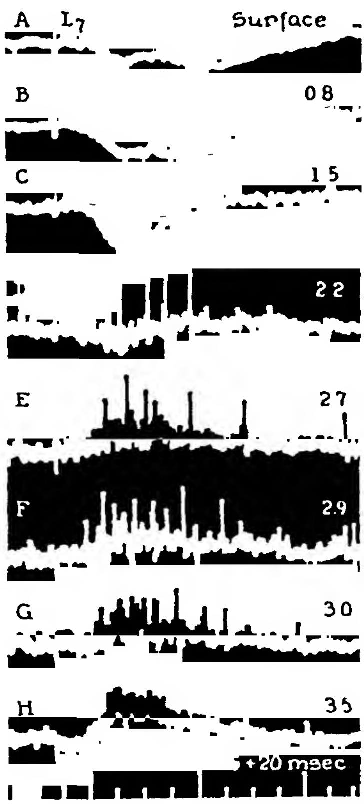

FIG. 6. Long spinal reflex. Activity recorded by microelectrode in the L7 segment. The figures at the right give the depth of the recording tip below the surface. Note the slow potential positive in the dorsal regions and negative in the ventral regions of the cord, also localization of the active neurons revealed by the occurrence of spike potentials.

FIG. 7. Records obtained by microelectrode in the L5 dorsal (A, B, C, D) and ventral (E, F) horns. A, C and F responses to L7D.R. volley. B, D and E responses to brachial plexus volley. Convergence occurs only in the ventral horn. Further description in text. The two long spike potentials in E may represent motoneuron activity.

the pool subject to inhibition by the long spinal reflex impulses. At the same time, by direct inhibitory action of its own, the posterior L7D.R. volley must remove from the responsive system those motoneurons that contribute to the test response employed in 5A, and which can be inhibited by the action of the long spinal reflex impulses. Whatever may be the unique explana-

tion of observations such as those of Fig. 5, it is well to remember that the absence of any change in a two-neuron-arc reflex test does not preclude the occurrence of inhibitory action by the conditioning activity. The occurrence of an inhibitory action can be denied, and then with some reservations, only after an exhaustive search has been made with uniformly negative results.

Responses of the gray substance. Following a single shock to the brachial plexus and the resulting discharge into the descending propriospinal system, certain pools of neurons within the lumbar enlargement become active. The pools of active neurons are almost, if not entirely, confined to the ventral horn.

Figure 6 shows a series of records made at various depths in the dorso-ventral axis of the spinal cord extending from the dorsum to the ventral margin of the ventral horn. The recording microelectrode was inserted from a point 1 mm. medial to the root entry line and therefore passes just medial to the heart of the gray substance. Records A, B and C of Fig. 6 present typical results obtained when the microelectrode tip lies on the dorsal surface, in the dorsal column or dorsal horn. The dorsal regions of the cord become positive as a result of the brachial plexus shock, for a period of time approximately coextensive with the facilitation period of the motoneurons (cf. Fig. 15, 16). The neurons of the dorsal horn do not become active, a fact which has repeatedly been confirmed. For example, in Fig. 7 dorsal horn units of the L5 segment are seen to respond in A and C to a single L7-D.R. shock. These dorsal horn units, however, are quiescent during the activity period resulting in B and D of Fig. 7 from a brachial plexus shock. The dorsal horn cannot, therefore, be concerned in a primary sense with long spinal reflexes. Occasionally, if the hind limb movement caused by the long spinal reflex action be great, a small burst of dorsal horn activity may result. When this occurs, it does so with a latency of approximately 30 msec., which fact clearly defines it as a rebound from the periphery.

As the recording microelectrode passes from the intermediate region through the ventral horn, nuclear discharges are written on a slower negative potential which appears to increase in amplitude as the microelectrode approaches the ventral margin of the ventral horn. When the margin is reached, the nuclear discharges fade out, leaving the slower negative potential in prominence (Fig. 6H). Units within the ventral horn that are open to activation by the sequelae of a brachial plexus shock may also be available to local dorsal root volleys. An example of this observation is presented in Fig. 7, in which E shows the discharges of two types of units in response to a brachial plexus volley, while F reveals the smaller units unmistakably responding again as the result of a local dorsal root shock. These experiments indicate that whatever convergence of long and short spinal reflexes occurs, must take place within the ventral horn.

The more lateral regions of the ventral horn are also active following brachial plexus stimulation. Figure 8 (A to G) presents records obtained in the lateral part of the ventral horn of the L5 segment. The microelectrode

is placed at successive positions from 2.5 to 3.1 mm. below the dorsal surface of the cord. Figure 8H was recorded in the L6 segment, 3.2 mm. below the dorsal surface and 0.7 mm. lateral to the root entry line, to demonstrate the

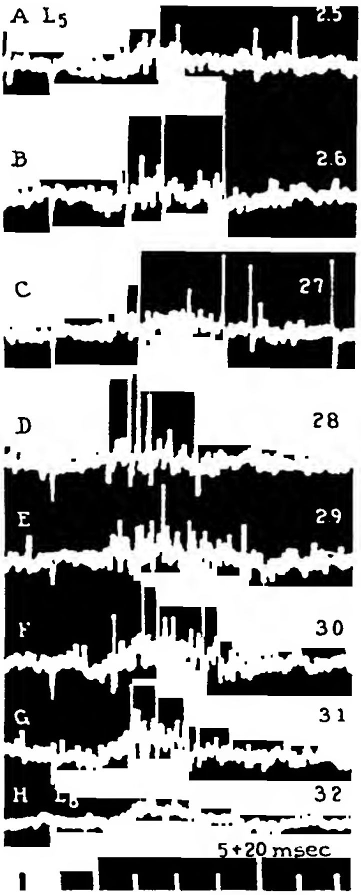

FIG. 8. Long spinal reflex activity recorded from the lateral part of the ventral horn in the L5 segment. The figures at the right give the depth of the recording tip of the microelectrode. H, record lateral to the ventrolateral angle of the ventral horn in the L6 segment.

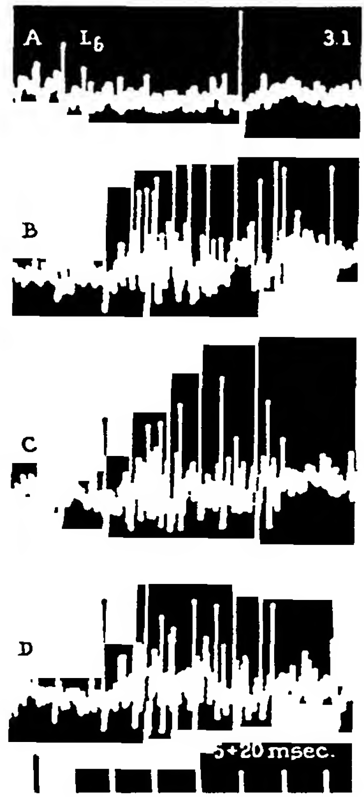

FIG. 9. Records from the medial aspect of the ventral horn in the region of the *nucleus cornu-commissuralis anterior*. A, blank sweep. B, C, D, all similar.

presence of the slower negative potential at the lateral margin, as well as at the ventral margin of the ventral horn.

The most prominent discharges to be found in the lumbar spinal cord, on stimulating the brachial plexus, occur at the medial aspect of the ventral horn. The records of Fig. 9 were all obtained in the L6 segment, 1.4 mm. medial to the root entry line and 3.1 mm. below the dorsal surface. Observa-

tion A shows the level of activity maintained in the absence of specific stimulation, whereas B, C, and D (all similar) illustrate the profusion of activity elicited by a single brachial plexus shock. These powerful discharges are of particular interest, for they are found characteristically in this area heavily endowed with commissural neurons (*nucleus cornu-commissuralis anterior*). That the activity of commissural neurons is so conspicuous will not be occasion for surprise when the extent to which crossed activity participates not only in contralateral reactions, but also in "purely ipsilateral" reactions is appreciated (cf. Fig. 15, 16).

In general it may be stated that the gray substance of the side contralateral to that receiving the afferent brachial plexus volley becomes active in a pattern entirely comparable to that which has been described for the ipsilateral gray substance.

With the examples of nuclear activity presented in Fig. 6, 7, 8 and 9 considered together, certain conclusions may be reached. Virtually all regions of the ventral horn exhibit activity as a result of brachial plexus stimulation, whereas the dorsal horn and most, if not all, of the intermediate region remain silent. This fact is worth noting in interpreting the sign of the slow potentials, such as those recorded in Fig. 6. These potentials are essentially similar in nature to the intermediary potentials discussed by Hughes and Gasser (1), with the outstanding difference that the polarity is reversed in the dorso-ventral axis. The reversed sign is to be correlated with the fact that, with brachial plexus stimulation, activity in the lumbar enlargement is centered in the ventral horn, while under the conditions of local afferent stimulation intense activity is engendered in the dorsal horn.

Nuclear activity begins 5 to 7 msec. after the afferent stimulus, as evidenced both by the slow potentials and by the earliest recorded spike potential activity. During the interval between the 10th and the 20th msec. after the shock, nuclear activity is most intense, following which reversion to the resting state occurs, the most prolonged discharges usually ceasing within 35 to 40 msec. The onset of nuclear activity, therefore, coincides with the beginning of facilitation in the motor pools, and the two events follow parallel subsequent courses. One may conclude that the local internuncial pools, rather than the tract fibers, are primarily responsible for excitation in the motor nuclei. It follows then that the tract fibers are rather more liberally distributed to the internuncial pools of the ventral horn than to the motoneurons, at least insofar as their end-action is excitatory.

On many counts the discharges illustrated in Fig. 6, 7, 8 and 9 represent preponderantly the activity of interneurons and not that of motoneurons. For instance, evidence may be found in the fact that unitary discharges such as illustrated precede by a distinct interval the onset of motoneuron discharge, while the subsequent time-intensity course parallels, not motoneuron discharge, but motoneuron facilitation as measured by test two-neuron-arc discharges. In some experiments in which motoneuron discharge is copious, this discharge appears at the microelectrode as a deep positive

potential cutting into the heart of the more prolonged negative potential. In other experiments the anatomical location of the microelectrode minimizes the possibility of motoneuron contribution to the record, as in Fig. 9. The size of the recorded spike potentials is in some measure a criterion, most of the spike potentials recorded in the present experiments being no more than several hundred microvolts in amplitude, whereas large neurons contribute spike potentials of 2 or more millivolts (cf. the solitary cells of the dorsal horn, [4]). Finally it is a common experience that unitary discharges referable to motoneurons are notoriously difficult to record, for the motoneurons are usually almost immediately damaged by the approaching microelectrode.

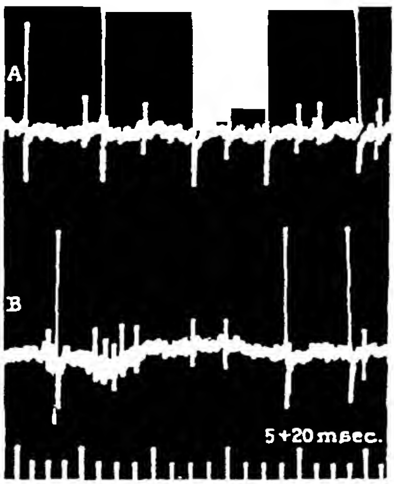

FIG. 10. Records from the ventral horn showing in A, the spontaneously occurring discharges of two units, one yielding the large spikes, the other small spikes. In B, the large spikes are suppressed, the small spikes are multiplied by long spinal reflex activity. The neurons yielding these spikes are reciprocally related under the conditions of long spinal reflex action.

On such occasions a furious injury discharge is encountered, the individual spike potentials of which are several millivolts in amplitude. On the contrary, the responses pictured in Fig. 6, 7, 8 and 9 are easily and regularly obtainable. It is possible that the two large spike potentials appearing in Fig. 7E represent motoneuron discharges, for they occur with appropriate latency and are of large size.

Inhibition at internuncial levels—reciprocal innervation. Neurons within the ventral horn are not always brought into activity subsequent to brachial plexus stimulation; occasionally neurons are found, by an exploring microelectrode, in which activity is suspended rather than initiated. For example, in Fig. 10A there are two types of units responding spontaneously and yielding characteristic recorded spike potentials. The microelectrode for these observations was placed 2.8 mm. below the dorsum, at the root entry line of the lower part of the L5 segment, *i.e.*, in the ventral horn. For record 10B a single shock was delivered to the brachial plexus. The responses identified by the smaller action potentials are strongly intensified, whereas the larger spike potential responses are suspended for a period in excess of 50 msec.

The two units clearly are reciprocally related, under the conditions imposed by long spinal reflex activation.

Interaction between long spinal reflexes and local reflexes of more than two neurons. Reference to Fig. 4 shows that the discharges through arcs of more than two neurons in the segmental reflex are not affected until the shock intervals used in records 4N and 4O are reached. There is then a decrease in the discharge pertaining to these multineuronal arcs. In the case of the segmental reflex it might be said that such a decrease in higher order discharges is due to occlusion by the simultaneously facilitated antecedent two-neuron-arc discharge. This explanation cannot be ruled out, as there

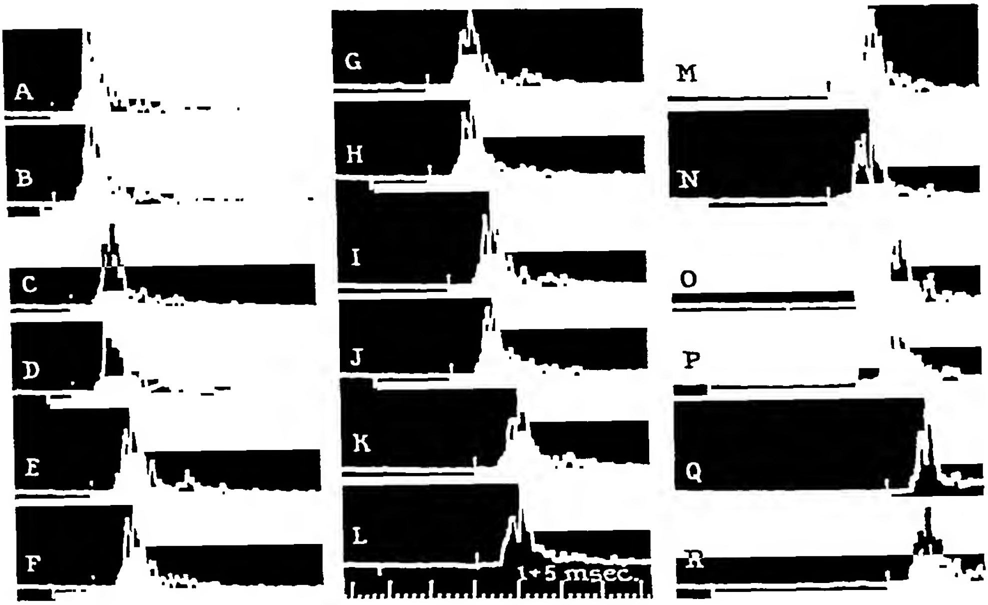

FIG. 11. Interaction of long spinal reflex and short spinal reflex having as its shortest path one of more than two neurons. Control records of the short spinal reflex (A, C, E . . .) are alternated with observations on interaction (B, D, F . . .). The motoneuron discharge evoked through the long spinal reflex paths is very small, but can be identified in L, N, P and R.

is every indication that the finer structure of the reflex system is such as to permit this to occur. To study internuncial interactions it is desirable to devise test reflexes unencumbered by two-neuron arcs.

In Fig. 11 a reflex consisting of the discharge elicited among S1 motoneurons by a L6D.R. shock is placed at various intervals during the course of the activity evoked by a brachial plexus shock. At no time are two-neuron arcs involved in the reactive systems, so that activity in the higher order arcs is not thereby obscured. The testing L6 to S1 reflex is somewhat variable. Accordingly controls are alternated with conditioned responses, so that a fair estimate of interaction may be attained. In this experiment, motoneuron discharge in response to the brachial plexus stimulation in

isolation is insignificant. No tangible interaction occurs before an interval of 14 msec. separates the two shocks (11N). At this interval the earliest discharges of the local reflex appear to be favored over the later discharges. It is known that internuncial activity and motoneuron facilitation begin about 6 to 7 msec. after the delivery of a brachial plexus shock. The mild facilitation of higher order arcs, seen in 11N and 11P, takes place only at the peak of the facilitation period of motoneurons as revealed by two-neuron arcs (at 15 to 20 msec., cf. Fig. 15, 16). It is most likely, therefore, that the higher order arcs are influenced under the circumstances at the final relay, although on the basis of the observations of Fig. 7 (E and F) some interaction at interneurons of the ventral horn may be expected. It is probable that the greatest part of the local reflex pathways relay in the dorsal horn

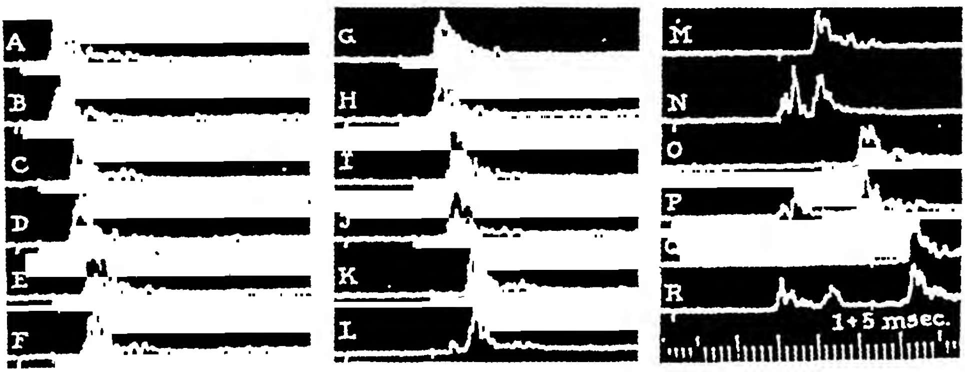

FIG. 12. Experiment similar to that illustrated in Fig. 11, but from another preparation in which the long spinal reflex discharge is ample. Note that the short spinal reflex obliterates the long spinal reflex discharge, but does not in turn suffer complete inhibition. The interaction between these two reflexes is asymmetrical.

and the intermediate nucleus of Cajal, rather than in the ventral horn, for if the converse were true one would expect to find significant changes in the short spinal reflex occurring long before they do, perhaps even as early as 2.5 to 3 msec. after the brachial plexus shock. It will be recalled in this connection that facilitation of local reflexes at the intermediate nucleus is the primary event when the pyramidal system forms the source of conditioning activity (4).

More striking than the allied or reinforcing actions of long spinal reflexes on local reflexes of higher order are the antagonisms between these two classes of reflex. In Fig. 11 it will be seen that the discharge area of the local reflex is slightly decreased in P and R as a result of the long spinal reflex activity.

Figure 12 presents additional features in the interaction of long and short spinal reflexes. It is constructed in the same way as Fig. 11, utilizing the same reflex pathways, but it is taken from another experiment in which the motoneuron response to the brachial plexus stimulus is of considerable

size. This response is seen in the first discharge grouping in Fig. 12R; the second grouping belongs to the short spinal reflex. It will be seen from observations A to J of Fig. 12 that the short spinal reflex discharge in simultaneous combination with (J), or preceding the long spinal reflex discharge, obliterates the latter. On the other hand, as the short spinal reflex falls later within the long spinal reflex discharge period (L and N) or follows after that period (P and R), it is in turn but little affected. The interaction between these reflexes then is far from symmetrical.

Observations of particular interest have been made in several experiments by causing the two reflex discharges to fall in simultaneous combination, as in Fig. 12J. The effect, to be described in greater detail in connection with Fig. 13, is the more striking when the long spinal reflex discharge is

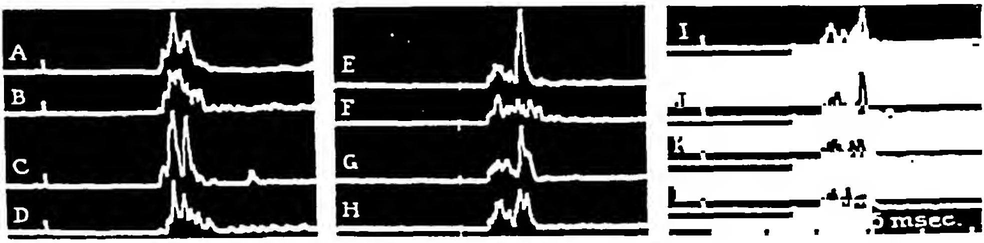

FIG. 13. Mutual inhibition of long and short spinal reflexes. The shock interval is adjusted so that the two reflex discharges fall in simultaneous combination. A–D, long spinal reflex. E–H, short spinal reflex. I–L, combined reflexes.

sizeable. In Fig. 13 are presented the results of one such experiment. A brachial plexus shock and an L6D.R. shock are set at such an interval that the motoneuron discharge periods of the two reflexes coincide in time. On account of the variability of response, four sets of responses to the shocks in isolation and together are reproduced. The shocks are separated by approximately 9 msec. Records A, B, C, and D of Fig. 13 show the response elicited by the brachial plexus shock alone, while E, F, G and H show the response to the L6 shock alone. In I, J, K and L the two shocks are delivered in succession at the predetermined interval. It is obvious that the response to the combined stimulation is much less than the sum of the responses to either reflex stimulation alone. What is more, all of the responses elicited by the two shocks in concert are smaller than any of the responses evoked by either of the two shocks in isolation. The effect illustrated in Fig. 13, therefore, is too great to be accounted for in terms of occlusion; there is actually a mutual inhibition between the two reflexes.

It is apparent in the observations of Fig. 11, 12 and 13 that the long spinal reflex has a feeble inhibitory action on the short multineuronal reflex discharge, and that this action is virtually independent of the size of the motoneuron discharge evoked through the long spinal reflex pathways. It is improbable, therefore, that motoneuron activity, as the final link of the long spinal reflex, is a factor contributing to the observed inhibition of the

short spinal reflex. It is known from Fig. 10 that some interneurons of the ventral horn are inhibited by the brachial plexus volley, and undoubtedly some motoneurons are inhibited for a similar period of time.

The evidence for direct inhibition is largely derived from conditioning curves such as those seen in Fig. 5A, 15, 16. The response to the test volley measures the average excitability of a selected pool of motoneurons. It does not and cannot reveal whether the motoneurons which are inhibited initially are subsequently excited, and contribute therefore to the facilitation phase of the conditioning curve; or whether they remain inhibited throughout, taking no further part in the cycle of events, while other individuals of the motoneuron population are recruited into the test discharge. Since the latter is manifestly true for interneurons (Fig. 10), there is no reason to suppose that it is not also valid for motoneurons.

It has been noted that the long spinal reflex discharge is relatively variable. When this discharge is smaller than that of the short spinal reflex, its obliteration by the short reflex might be explained by occlusion. However, in view of the asymmetry of interaction between the two reflexes (Fig. 12) and the magnitude of the inhibitory effect exerted by the short spinal reflex (Fig. 13), blocking as it does discharges greater than itself, occlusion cannot adequately account for the results. There is fortunately a good deal of evidence to show that dorsal root volleys through the L6D.R. exert a direct inhibitory action on motoneurons of the S1 segment as tested by two-neuron-arc discharges.

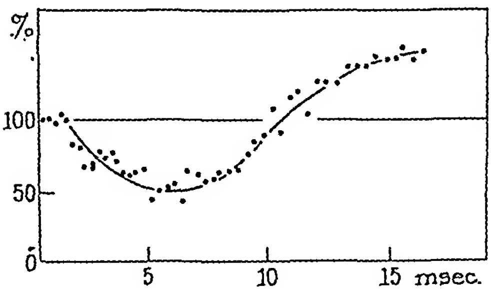

FIG. 14. Inhibition of some motoneurons of the S1 segment as evoked by L6 dorsal root volleys and tested by a two-neuron-arc discharge. The latency for inhibition in this example is *ca.* 1.5 msec.; other S1 motoneurons are facilitated with the same latency (cf. 3, Fig. 2). The motoneuron discharge of the S1 pool to L6 shocks occurs within the period during which these motoneurons are inhibited and the others are facilitated. Further description in text.

In the original description of direct inhibitory action (3) inhibition lasted for only 1 to 1.5 msec., thereafter being supplanted by facilitation. Although this is a frequent finding, some experiments by virtue of a different selection of testing two-neuron-arc discharges reveal that inhibition of some motoneurons within the S1 pool is maintained throughout the time during which others are discharging in response to the L6 volley. Figure 14 illustrates an experiment of this type. Amplitude of the two-neuron-arc test discharge is plotted as a function of the interval between the conditioning and the testing shocks. The motoneurons tested belong to the S1 segmental pool. No change in the test discharge appears during the first 1.5 msec., although "masking of inhibition" may account for this. For the next 10 msec., during which a few S1 motoneurons are discharging, others involved in the two-neuron-arc test are inhibited. The effect illustrated in Fig. 14 would account for the

suppression of the long spinal reflex discharge, given an appropriate selection of motoneurons by the long spinal reflex.

The interaction between long and short spinal reflexes, as presented in Fig. 11, 12 and 13 is one of considerable complexity. It seems certain that the results of these experiments represent in large measure the interplay of direct inhibitory actions. The two reflexes, long and short, are preponderantly though not exclusively antagonistic. The antagonism, moreover, is not basically one of competition for pathways; indeed, the present experiments point to a rather high degree of independence between the paths occupied by the two reflexes, at least insofar as excitatory actions are concerned. Until the mechanism of direct inhibition and the substrate of finer

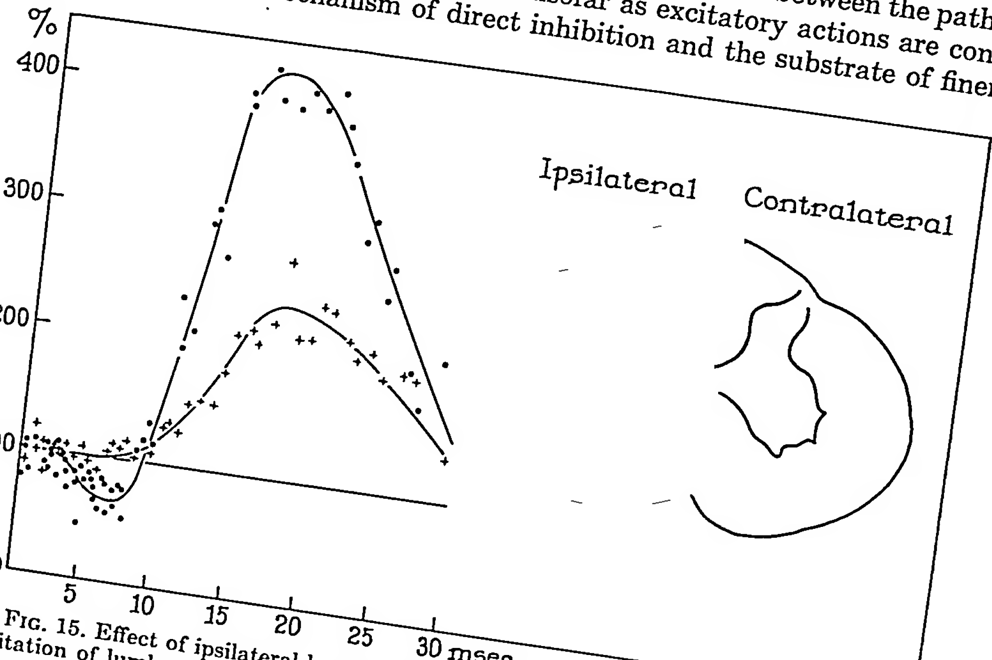

FIG. 15. Effect of ipsilateral hemisection in the upper lumbar cord on inhibition and facilitation of lumbar two-neuron-arc discharges by brachial plexus volleys. On the left are conditioning curves obtained before (dots) and after (crosses) hemisection. At the right is shown the extent of the lesion as determined from serial sections.

anatomy underlying that form of inhibition are better understood, no statement on inhibitory convergence can have more than the chance validity of an *ad hoc* argument.

The interaction between long spinal reflexes is quite different from that between long and short spinal reflexes. In brief, facilitation of motoneuron discharge is the final result, whether the successive long spinal reflexes both originate from the same side of the body, or whether they arise from opposite sides.

Tracts mediating long spinal reflex activity—effect of partial lesions of spinal cord. The impulses which link the cervical and lumbar enlargements during the course of long spinal reflex activity are so diffuse and dispersed that the

direct experimental approach by the use of microelectrodes has failed to yield significant results. These have been obtained, however, by resorting to a study of the effect of spinal cord lesions upon the course of facilitation and inhibition of lumbar motoneurons by brachial plexus shocks. The discharge of motoneurons has not been used because of the variability of response.

A hemisection, ipsilateral with respect to the brachial plexus stimulation and the tested motoneurons, abolishes the early inhibitory period, but does not similarly abolish the period of facilitation. Figure 15 illustrates this finding. On the left of Fig. 15 are drawn the conditioning curves of a segmental two-neuron-arc discharge, amplitude of the test response being plotted as

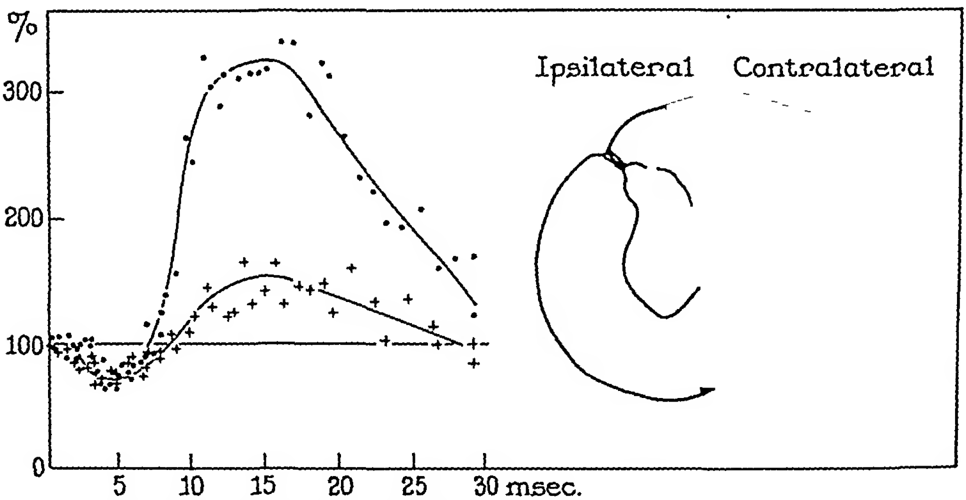

FIG. 16. Effect of contralateral hemisection in the upper lumbar cord on facilitation of lumbar two-neuron-arc discharges by brachial plexus volleys. Note that inhibition is not impaired by contralateral hemisection. On the left are conditioning curves obtained before (dots) and after (crosses) hemisection. The diagram at the right illustrates the extent of the lesion.

a function of the interval by which the test shock follows the brachial plexus shock. The curve represented by dots is constructed from observations obtained before hemisection, that represented by crosses from observations following hemisection. The extent of the lesion is shown on the right, as determined from serial sections. The drawing is made by projection, and the full extent of the lesion is found by recording the limits of the lesion as they occur in various sections. The lesion in Fig. 15 is actually slightly greater than a hemisection, as it transgresses the mid-line in the dorsal column and at the base of the ventral column. A comparison of the curves before and after ipsilateral hemisection shows that the paths mediating the direct inhibition are severed, whereas only a fraction of the excitatory paths is blocked.

Figure 16, constructed in the same way as Fig. 15, illustrates the effect of contralateral hemisection. The lesion in this case is complete on the contralateral side, includes about a third of the dorsal column on the ipsilateral

side, and damages some fibers close to the ventromedian fissure on the ipsilateral side. Other experiments (Fig. 17) have shown that involvement of the dorsal column is without significance, so that for practical purposes this lesion may be considered as a hemisection. Consideration of the conditioning curves in Fig. 16 shows that inhibition is not affected by contralateral hemisection, while facilitation is severely depleted although not abolished.

The results of Fig. 15 and 16 taken together indicate that the fibers mediating inhibition enter the lumbar enlargement with strict regard for laterality. On the other hand, the pathways mediating excitatory effects arising on one side of the body and exerting their action, under the conditions of the experiments, on that same side of the body, are not only ipsilateral and uncrossed, but are doubly crossed as well.

Similar effects are noted when the object of study is the crossed long spinal reflex. Thus lesions either ipsilateral or contralateral with respect to the stimulated brachial plexus deplete the facilitation of contralateral (crossed) two-neuron-arc discharges. Despite a number of attempts to uncover direct inhibition of two-neuron-arc discharges on the side contralateral to the stimulated brachial plexus, this has not been observed. It is believed not to occur.

The fact that the intraspinal pathway mediating direct inhibition is strictly unilateral (Fig. 15, 16), taken together with the observations on latency of the direct inhibitory action (Fig. 4, 5, 15, 16), confirms the view that the long propriospinal fibers themselves exert the inhibitory action. It will be remembered in this connection that Sherrington and Laslett (8) found no evidence to suggest that the long propriospinal fibers decussate. There seems to be no reason *a priori* why long propriospianl fibers arising in the cervical enlargement on the side contralateral to a given brachial plexus volley should not be activated by commissural neurons intrinsic to the cervical enlargement. Subsequently, and maintaining laterality, these propriospinal fibers could achieve an inhibitory effect on lumbar motoneurons on the side contralateral to the brachial plexus volley. The evidence on hand that contraindicates such an eventuality as this is of necessity inconclusive.

Lesions confined to the dorsal columns, of which one example is illustrated in Fig. 17A, do not result in a deficit in facilitation of two-neuron arcs in the lumbar enlargement. This is taken to indicate that aborally directed fibers of the dorsal columns do not participate to a significant degree in mediating long spinal reflex activity under the present conditions of experiment. Superficial lesions of the ipsilateral lateral column (Fig. 17B) have but a slight effect. When the ipsilateral lateral column is more seriously involved (Fig. 17C) a serious deficit in facilitation follows. Hence the ipsilateral column, and especially its deeper parts, is important for the mediation of ipsilateral excitatory effects.

The contralateral half of the spinal cord is important in mediating "ipsilateral" reflex effect, and the contralateral ventral column appears to contain

the majority of the fibers contributing to "doubly crossed" excitation. For instance, Lesion 17H, severely damaging the contralateral lateral column, is without effect on the ipsilateral reflex excitation. Comparison of Lesion 17C and Lesion 17H, these being virtually symmetrical lesions, leads to the conclusion that the lateral column is in the main reserved for truly unilateral ipsilateral transmission, *i.e.*, the intrinsic fibers of the lateral columns do not measurably supply the commissural nuclei. In this connection it is of interest

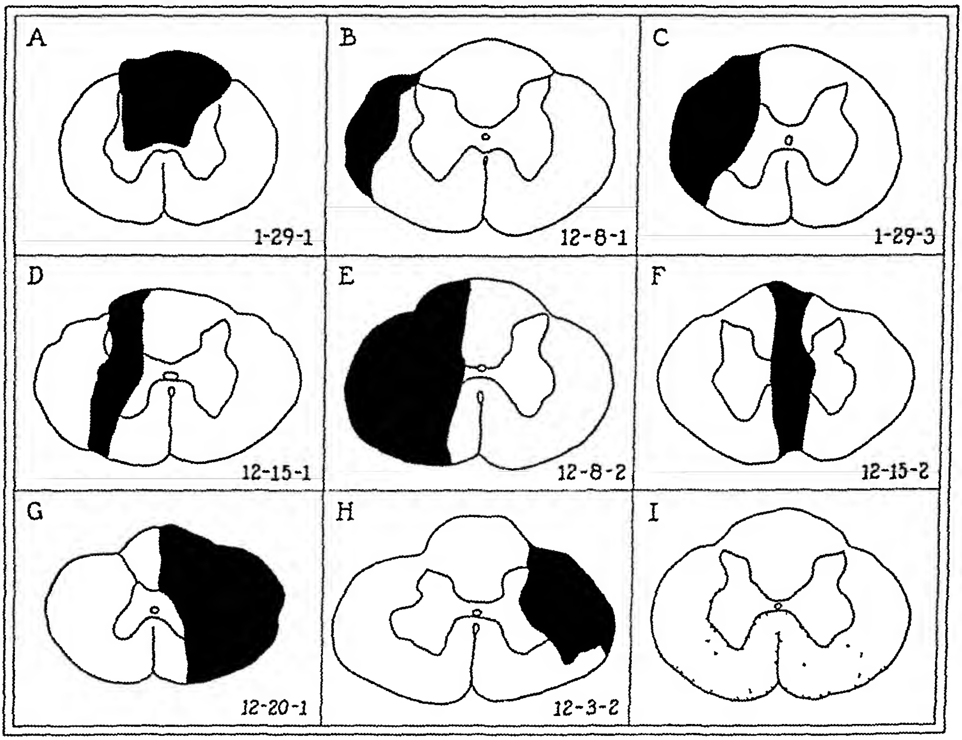

FIG. 17. A–H, various lesions of the spinal cord the effect of which on long spinal reflex action is discussed in the text. Lesions C and H are virtually symmetrical as are E and G. I, diagram of the cross-sectional area of the cord. The shaded area contains the tract fibers, ipsilateral and contralateral, which contribute to "purely ipsilateral" end effect. In essence the lateral columns carry unilateral tracts, the ventral columns bilateral tracts.

to recall the important part played by the lateral column in such exquisitely unilateral reactions as the scratch reflex (8).

All lesions transgressing on the ventral columns, ipsilateral or contralateral diminish the intensity of long spinal facilitation in varying degree (Lesions 17D, E, F, G). Given approximately symmetrical lesions of the ventral columns (Lesions 15 and 16, Lesions 17E and 17G), the contralateral lesion appears to violate facilitation to the greater extent. This observation may depend upon the fact that the inhibitory actions of brachial plexus volleys are pre-eminently ipsilateral. Accordingly, contralateral lesions would de-

plete only excitatory barrage mediated through commissural neurons, while ipsilateral lesions would diminish inhibition along with excitation. In the former case the residual excitatory barrage might be pitted against the full inhibitory power of the reflex activity; in the latter case the contralateral barrage would be relatively unfettered by inhibition.

Diagram I of Fig. 17 illustrates the areas contributing descending fibers for the transmission of ipsilateral long spinal reflex excitatory effect. It is constructed according to the information gained from experiments involving lesions, including those illustrated. Emphasis must be placed on the view that the present experiments, in which long spinal reflex pathways are activated in mass, tend to delineate the overall dimensions of the system, rather than the dimensions of the system as utilized for the performance of any appropriate reflex act. Obviously, localizations within the system exist for the transmission of impulses involved in the performance of certain acts, the scratch reflex being a case in point. In fact, some localization is revealed in the present experiments, inasmuch as the cross-sectional area of the cord contributing fibers for the realization of unilateral end effect is asymmetrically bilateral, whereas the fasciculi proprii of the cord considered as a whole are bilaterally symmetrical.

In discussing the effect of lesions on the descending spinal reflex activity, it has been assumed that the action of such lesions is to interrupt or not, as the case may be, fibers conveying descending tract impulses, and there is little doubt but that this assumption is substantially correct. However, it must be remembered that postbrachial lesions of the ventrolateral columns affect decerebrate rigidity and stretch reflexes in the brachial field (7). It might be that a hemisection, for example, by virtue of some action analogous to the Schiff-Sherrington phenomenon, affects the number and pattern of descending impulses above the lesion as well as below.

Functional organization of the long spinal reflex mechanism. Figure 18 presents in diagrammatic form a survey of the functional connections established between the descending tracts subserving long spinal reflexes, interneurons and motoneurons of the lumbar enlargement. There is fragmentary evidence in the anatomical literature to show that neurons in the cervical cord which possess descending axons, lie in the depth of the ventral horn. The descending axons, at least in the upper lumbar region, lie in the lateral and ventral columns (Fig. 17). In Fig. 18, then, collaterals of the tract fibers are pictured as entering the two ventral horns from the lateral and ventral columns of the side ipsilateral to the afferent stimulation, and from the ventral column alone on the contralateral side (Fig. 15, 16, 17). The tract collaterals end on interneurons through the ventral horn, some being unilateral, others commissural (Fig. 6, 7, 8, 9). For purposes of description the unilateral interneurons are grouped together as the *nucleus proprius cornu anterioris* (N.Pr.C.A.), regardless of whether the functional grouping has a true anatomical entity as a nucleus or not. Commissural neurons are grouped into the *nucleus cornu-commissuralis anterior*, for which there is considerable functional and anatomical justification.

The first tract impulses reach the lower spinal mechanism at 2.5 to 3 msec. after the brachial plexus shock, and act in a way so as to result in inhibition of certain two-neuron-arc reflexes. After the lapse of another 3 to 5 msec. interneurons within the ventral horn become active, and it is at this time that the first excitatory action on motoneurons has been detected. It is only after an additional 2 to 3 msec. that the motoneurons in turn discharge impulses to the periphery. The time interval that exists between the arrival of excitatory impulses within a given nucleus and the discharge of impulses

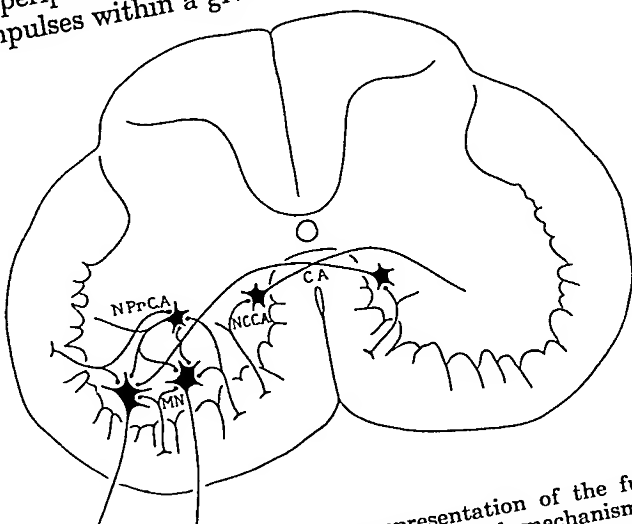

FIG. 18. Diagrammatic representation of the functional organization of the lower spinal mechanism to illustrate the connections established by long spinal reflex action. C.A., anterior commissure; M.N., motoneurons; N.C.C.A., nucleus of the anterior commissure; N.Pr.C.A., internuncial nuclei of the ventral horn.

from that nucleus has been designated as nuclear delay. In some situations, notably at the motoneurons, nuclear delay can be measured, in others it remains conjectural. The same, of course, can be said of synaptic delay. As more knowledge is gained of the dynamics of action within the central nervous system, particularly when nuclei are activated by diffuse discharges (occasioned by simple conduction dispersion or by antecedent nuclear intervention) rather than by highly synchronized volleys, it becomes increasingly clear that nuclear delay is a phenomenon of general significance. Central latency is the sum of central conduction and nuclear delays. All this, of course, does not explain nuclear delay. The simplest assumption is that it is the time necessary for the diffuse influx of impulses to reach an intensity sufficient to secure discharge from a nucleus. This assumption is undoubtedly valid for many nuclei under various conditions of activation, but it may not prove valid for all. It should be noted that the synaptic delay of Sherrington encompasses both the synaptic delay and the nuclear delay of contemporary description.

There is at the present time no clear picture of the anatomical substrate of direct inhibition. Consequently no adequate attempt is made in Fig. 18 to depict inhibitory connections.

Throughout the activity period engendered by brachial plexus stimulation, the dorsal regions of the cord remain silent and play no immediate part in long spinal descending activity. In this connection the contrast between the spinal mechanism as activated by the long spinal reflex system and by the pyramidal system (4, Fig. 14) is striking. The contrast emphasizes the nicety and precision with which various descending systems fractionate the spinal mechanism. At the present time there is little knowledge by which to decide whether the fractionation is predetermined by limitation of anatomical interconnection or whether it is brought about primarily by functional means.

Summary

Some aspects of the transmission of reflex effect from the forelimb to the hindlimb have been examined. Such transmission of necessity employs the descending propriospinal neurons, long and short, as a mediate system.

Transmission of activity arising on one side of the body involves propriospinal tracts of both halves of the spinal cord, as a result of the free yoking together of the brachial mechanisms. Free decussation occurs again in the lumbar cord. Consequently unilaterally evoked activity, by transmission through the propriospinal system, excites motoneurons by strictly unilateral paths, by decussating or crossed paths, and also reaches motoneurons of the same side through doubly decussated paths. The lateral columns are more intimately concerned with strictly unilateral transmission. The ventral columns subserve both unilateral and bilateral transmission. No significant functioning in the present experiments can be attributed to the dorsal column.

The first effect exerted in the lumbar cord by a brachial plexus volley is inhibition of some motoneurons of the ipsilateral side through unilateral pathways. The latency of inhibition allows only for conduction and a single synaptic relay in the cervical cord between primary afferent and propriospinal fibers. Hence long propriospinal fibers are involved and the inhibitory effect is a direct action.

Shortly after the onset of inhibition, interneurons of the lumbar enlargement become active and the motoneurons simultaneously are facilitated. At the height of the facilitation period, which lasts *ca.* 35 msec., motoneurons discharge impulses to the periphery. The long spinal reflex discharge is much more variable than short spinal reflexes, but under favorable conditions may approximate or even exceed the latter in size and synchrony.

In the lumbar enlargement the active neuron pools are confined to the ventral half of the cord. The dorsal horns and most, if not all, of the intermediate region remain inactive. In the ventral horn the most powerful activity is encountered in the *nucleus cornu-commissuralis anterior*, indicating the important role played by crossed mechanisms.

A degree of mutual interference takes place between long spinal reflex activity and short spinal reflex activity pertaining to multineuronal paths. This interference is designated as "mutual inhibition," for the deficit is frequently too great to be accounted for as occlusion. On the contrary, long spinal reflexes reinforce one another.

When nuclei are activated by diffuse discharges rather than synchronized volleys, synaptic delay is no longer the determining factor (along with conduction) in central latency. Nuclear delay, usually having several times the duration of the longest known central synaptic delays, becomes a characteristic feature of transmission and accounts for the greater part of central reflex time.

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INTERSEGMENTAL INHIBITION IN THE SPINAL CORD OF THE FROG

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It has long been known that in frogs exposed to cold a peculiar state of increased reflex excitability develops. (For the older bibliography see [2].) Most of the former investigations have been complicated by cooling of the whole frog by packing it in ice. Stimulation of the skin by cold and the tendency of cooled nerves to tetanic discharge may act to increase reflex excitability. However, Biedermann (2) has demonstrated that the increase of excitability occurs when these sources of error are avoided. He has interpreted the phenomenon as a change in the metabolism of the spinal cord during cooling. Von Baeyer (1) failed to observe any accentuation of the increased excitability of strychninized frogs as a result of cooling; on the contrary the reflex discharge appeared to be diminished. According to some older observations (1), strychnine tetanus may be entirely prevented by cooling.

Recently, Ozorio de Almeida (4–10) and his co-workers have published a series of papers on "epileptiform cramps" in the South American frog, *Leptodactyllus ocellatus*, induced when the isolated spinal cord (not separated from the vertebral column) was suddenly immersed in cold solutions but not when the solution was gradually cooled. Ozorio de Almeida *et al.* have described a "jump reflex" in the spinal frog elicited by allowing the frog to fall from a certain height on the sole of the foot and have shown it is increased by cooling.

Further to elucidate the effect of cold upon the central nervous system, we have used Winterstein's (11) isolated spinal cord preparation so that all interfering factors are avoided.

METHOD

The spinal cord of *Rana esculenta* was entirely isolated from the vertebral canal as far as the cauda equina and left connected by the sciatic nerve with the entire leg or with the lower parts only. The cord was immersed in oxygenated Ringer's solution, contained in a small cylindrical vessel, which was surrounded by a second cylinder of water to keep the Ringer's solution at any desired temperature. By this method, only the spinal cord and nerve roots were exposed to the change in temperature.

The ipsilateral reflex of one leg was elicited by electrical stimulation of the skin of the foot by fine wires which did not hinder movement of the leg. For registration of the reflex movement, the toes were connected by a thread to a straw lever writing on a smoked drum. To avoid fatigue the time interval between stimuli was rather long. In most experiments we employed a device producing a faradic current at a frequency of 15 per sec. and a duration of 1.5–2 sec. every 3 min.

Results and Interpretation

When the isolated spinal cord preparation was cooled down to temperatures of 12°–2°C. (in most experiments 8°–5°C.) there always occurred as an end result a *decrease* in reflex action, usually to the point of complete abolition

of excitability. In most experiments excitability was restored after raising the temperature to previous levels.

At the beginning of cooling the behavior of the various preparations was quite irregular. In more than half of the 38 experiments, a considerable initial increase of reflex response was observed before the onset of depression. The threshold for producing a minimal response, however, remained unaltered. This behavior was not influenced by changing the surrounding medium of the preparation (oxygenated-Ringer's solution or an atmosphere of pure oxygen). In order to decide whether the irregularity of results was due to differential cooling of various parts of the spinal cord, regional cooling by means

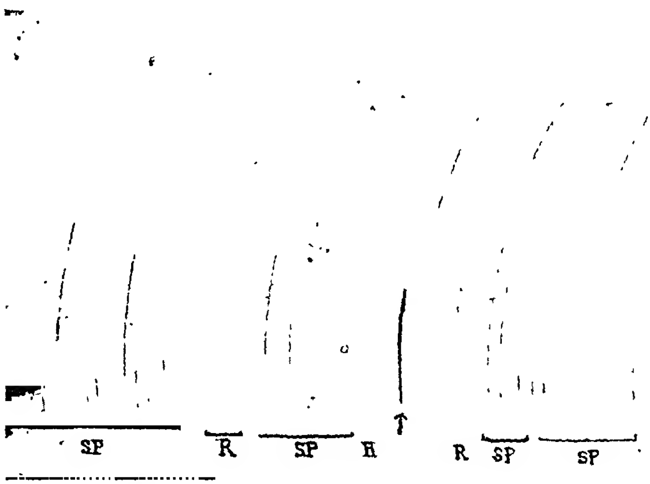

FIG. 1. Effect of cutting (↑) on the reflex (R) end the spontaneous (SP) movements.

of thermodes of cervical, dorsal, and lumbar regions was attempted. However no distinct differences could be established.

In spite of this negative finding several considerations suggested that the cause of the initial increase of reflex activity lies in the *removal by cold of some intraspinal inhibition.* Therefore the effect of section of the isolated spinal cord at various levels on the reflex activity of the segments below the section was investigated. An enormous increase in the magnitude of reflexes occurred in 17 of 19 experiments when the isolated spinal cord was sectioned at the cervical or upper dorsal level. This increase, however, was especially marked after section just above the lumbar enlargement. The threshold stimulus here too remained unchanged, which suggests that removal of an inhibitory influence normally exerted by the upper spinal segments on the lumbar segments is responsible for the phenomenon.

Cooling after sectioning the spinal cord at the lower thoracic level did not produce the initial increase in reflex excitability often observed when the whole isolated cord was exposed to low temperatures. The most convincing results on this point were obtained when the effect of cooling before and after

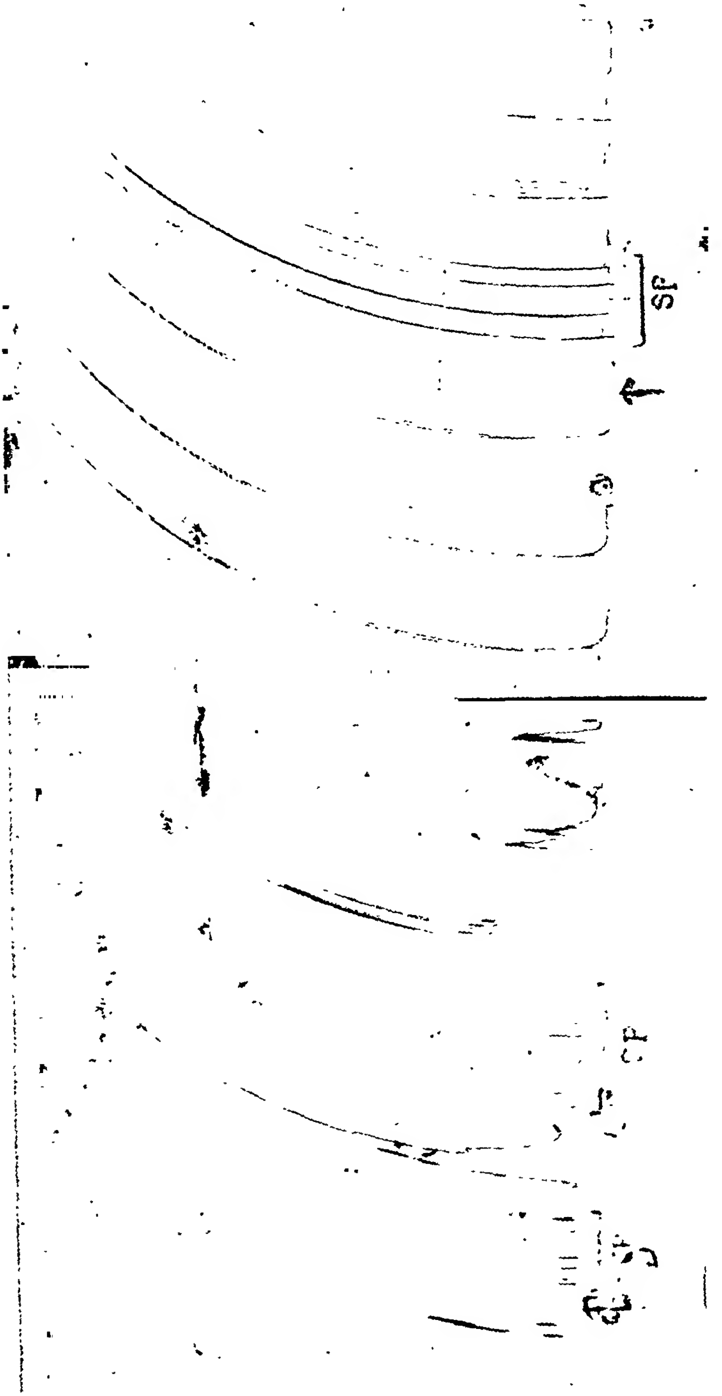

FIG. 2. Effect of cooling (↑) before (A) and after (B) section of the spinal cord on the spontaneous (SP) and the reflex (all not marked) movements.

cutting the spinal cord at the dorso-lumbar level was investigated. In 5 experiments an initial increase in reflex response in cooled preparations was observed before section of the cord. However, subsequent to the section an immediate diminution of reflexes resulted from cooling. Figure 2 demonstrates these effects and the enormous increase of reflexes following section is also to be seen.

These results suggest an interpretation of the behavior of the intact isolated cord to cooling. The initial increase of reflex excitability when the intact cord is cooled may be explained in the same way as the effect of sectioning the spinal cord. The effect of cooling may always be to reduce reflex activity; but cephalic segments exercising an inhibitory influence over lumbar reflexes may in some cases be the first affected by cold and thus their inhibitory action may be abolished. If this were true there would result an increase of reflex action which would persist until the lumbar segments in turn were inactivated by cold. Thus after the removal of the inhibitory mechanisms by cord sections only the depressing effect of cooling may be observed.

In addition to changes in induced reflexes from section and cooling of the spinal cord there are similar alterations in spontaneous movements. After cord section spontaneous movements also are increased in magnitude and frequency in some experiments. Cooling of the spinal cord acts similarly. In some preparations such movements did not occur at all until the cord was cooled. Spontaneous movements were observed not only in intact cord preparations but also after isolation of the lumbo-sacral segments (see Fig. 2). Analysis proves them to be of reflex origin. They disappear when the posterior spinal roots on both sides are sectioned. Their number may be greatly diminished or they may cease entirely after removal of the skin. The fact that these reflexes are augmented or appear only after cutting and cooling of the spinal cord proves that they are also subject to inhibition from cephalic cord levels. But since spontaneous and induced reflexes are independently and sometimes oppositely changed by cooling, the central mechanisms of the two may differ.

Conclusions

The effect of cooling and of sectioning the spinal cord of the frog when separated from the brain and removed from the body has been investigated.

1. Prolonged cooling of the spinal cord diminishes or abolishes ipsilateral reflex responses to electrical stimulation of the skin of the foot.

2. Diminution of reflex activity is often preceded by an increase.

3. Section of the spinal cord, especially just above the lumbar enlargement, regularly produces a marked increase of reflex response. The threshold strength of current remains unchanged in all three cases.

4. Cooling subsequent to low dorsal section always depresses reflex activity.

5. Spontaneous movements, proved to be of reflex origin, behave similarly to induced reflexes.

6. These observations are interpreted as follows: intersegmental inhibitory mechanisms involving the cervico-dorsal segments of the cord are affected by cold earlier than segmental reflex arcs of the lumbar region. Removal of such inhibition by cooling or by cord section increases the magnitude of reflex responses.

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OSCILLOGRAPHIC STUDIES ON THE SPINAL TRACT OF THE FIFTH CRANIAL NERVE*

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THE DEVELOPMENT of trigeminal tractotomy by Sjöqvist (40) for alleviation of facial pain brought about renewed interest in the anatomy and physiology of the spinal tract of the fifth cranial nerve. The tract has been studied clinically and experimentally by many workers, but there is disagreement concerning its role in mediation of sensations other than pain and temperature. Although the evidence is good that touch is mediated both by the chief sensory nucleus and by the spinal nucleus, there are many who still maintain that the spinal nucleus receives impulses elicited only by pain and temperature. Confusion exists also in regard to the relative location in the brain stem of fibers from the peripheral divisions of the trigeminal nerve and the caudal limits of their course.

Because of the preciseness of localization possible with oscillographic recordings within the brain stem, this technic was employed in studying the spinal tract. It was possible to record within the root the location of activity arising in each of the three divisions and to trace the activity to its caudal reaches.

MATERIALS AND METHODS

Twenty cats anesthetized with sodium pentobarbital were used in these experiments. A unipolar lead was oriented within the brain stem with the Horsley-Clarke instrument and electrical activity was recorded with a cathode ray oscillograph and loud speaker. The Horsley-Clarke instrument itself served as the reference electrode. Tactile responses were set up by stroking the skin and hair of the head with a camel's hair brush or blunt glass rod. Synchronous volleys were evoked by electrical stimuli from a thyratron stimulator. The frontal and maxillary nerves were suspended by hook electrodes and a shielded electrode was placed on the lingual nerve. Thus, branches of each of the three divisions of the trigeminal nerve were isolated for study. Impulses elicited peripherally were then traced into the brain stem in order to determine the central distribution of the primary neurones. Photographic records of the cathode ray screen during electrical stimulation and the appearance and sound of activity from tactile stimulation were then correlated with electrode positions as revealed by microscopic sections. The region under consideration was sectioned serially at 40μ, and every third section was stained by the Weil technic.

RESULTS

Upon tactile stimulation of the areas of distribution of the trigeminal nerve, electrical activity in the descending tract of the nerve resembles that characteristic of the activity in a peripheral nerve when its sensory field is stimulated. Tactile impulses originating in the ophthalmic division of the

* A preliminary report of these experiments was given before the American Association of Anatomists, New York, April, 1942.

This study was supported in part by a grant for equipment from the American Association for the Advancement of Science.

fifth cranial nerve pass into the ventral part of the spinal tract and may be traced caudally as far as the caudal third of the first cervical segment. From the mandibular division, similar impulses travel in the dorsal portion of the spinal tract as far as the junction of the medulla and the spinal cord. Potentials elicited by touch over the maxillary distribution were recorded in an intermediate position in the descending tract and were traced to the upper third of the first cervical segment. The fibers from each of the three divisions remain relatively discrete in the spinal tract. As the recording needle was lowered into the tract by 0.5 mm. intervals, tactile impulses appeared more or less discretely, first from the mandibular area, then from the maxillary,

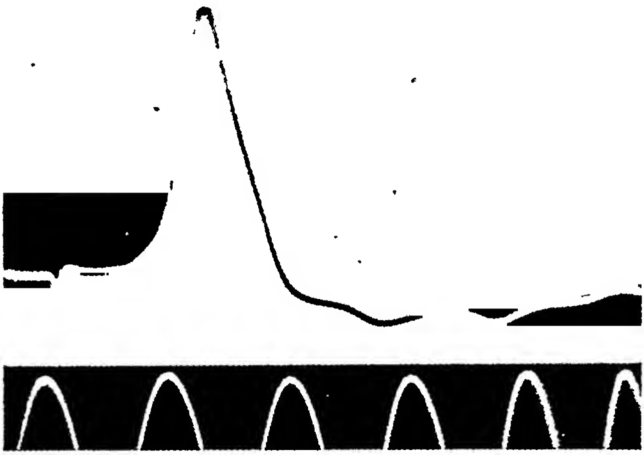

FIG. 1. Photograph of single sweep of cathode ray oscillograph; stimulation of peripheral branch of the trigeminal nerve (infra-orbital); unipolar recording from the spinal tract of the trigeminal nerve; time in msec. given by 1000-cycle wave. Positive potentials are recorded as upward deflections. Further details are given in the text.

and finally from the ophthalmic region. It is evident that in the cat large numbers of tactile fibers pass into the spinal tract of the trigeminal nerve. Tactile impulses may be traced almost as far caudally as a volley set up by a single electrical stimulus to a large peripheral branch of the trigeminal nerve, but, due to the fact that fewer fibers are active at any one time during tactile stimulation, there was uncertainty in delineating the extreme limits reached by those fibers. With the needle in the nucleus of the spinal tract, tactile impulses were recorded in the same dorso-ventral distribution as in the tract itself, although the intensity was less. The higher activity in the tract is due to the fact that at any given position of the needle there are not only fibers termininating medially in the nucleus of the same region but also fibers that continue caudally.

Following a single electrical stimulus to a branch of the fifth nerve, potential waves could be recorded in the spinal tract and nucleus. The action potential varied with the relation of the electrode tip to the spinal tract and its nucleus, and with conduction distance within the brain stem, but in general was made up of two waves each of which had one or more peaks. The first wave began 0.6 to 1.2 msec. after the stimulus, the time depending upon

the conducting distance, and was completed within 2 msec. In many cases, this wave appeared as a discrete spike, but frequently a second peak was recorded and was sometimes higher than the first. Figure 1 is a record from ex-

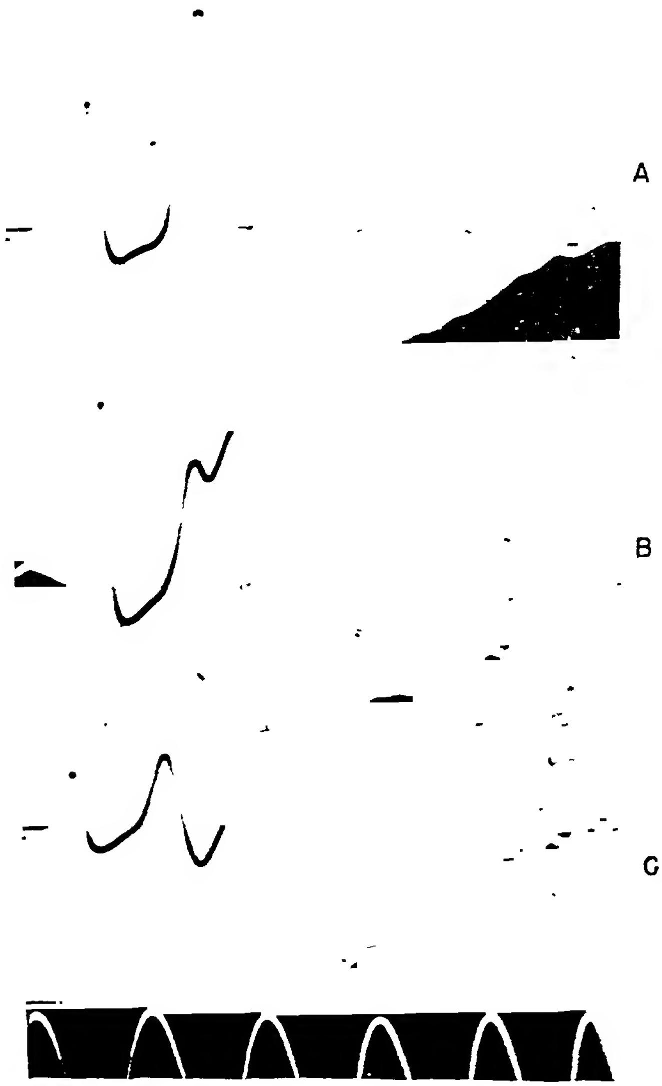

FIG. 2. Lingual nerve stimulated; other details as for Fig. 1.

periment 17 with the needle 13 mm. caudal to the entrance of the root. The action potential began at 0.65 msec., reached a peak at 1.3 msec., and ended at 2.0 msec. Figure 2A from experiment 18, with the recording electrode 3 mm. caudal to the entrance of the root, shows a slight break on the descending limb suggestive of an additional component. Figure 2C is a record ex-

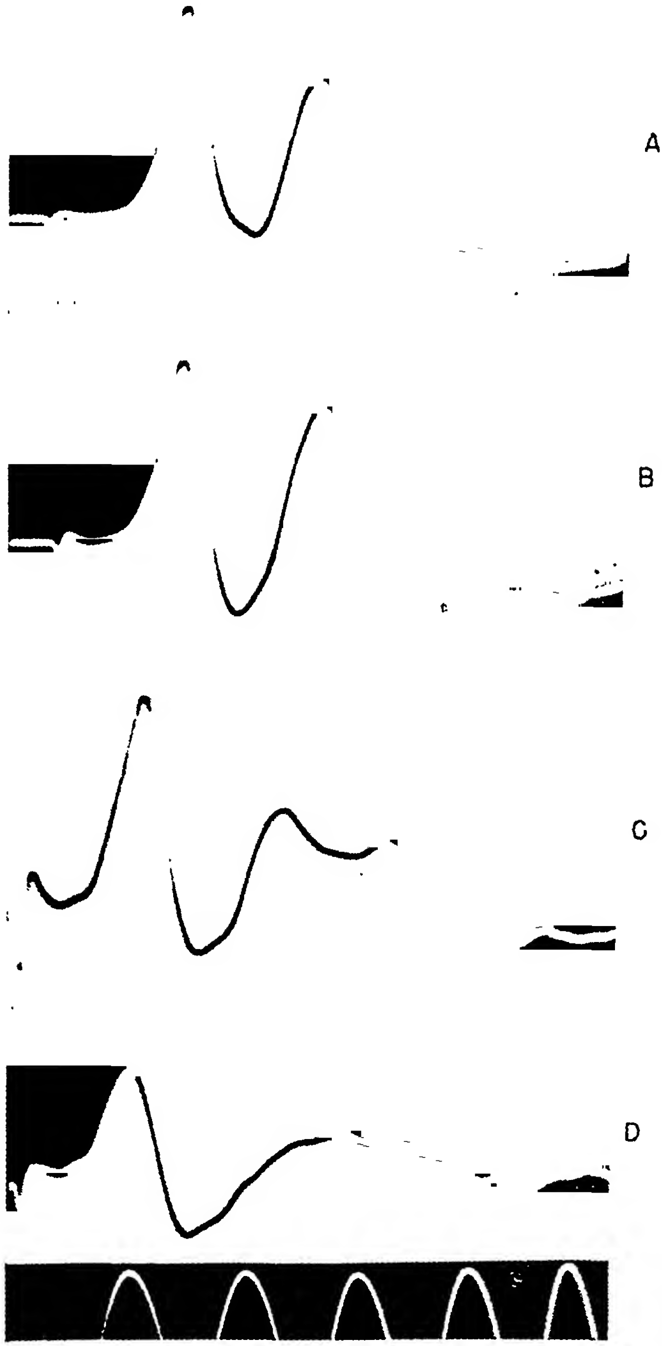

FIG. 3. Details as for Fig. 1.

hibiting two peaks and was taken one mm. dorsal and one mm. medial to that of Fig. 2A. The time course of the first peak was 0.65 msec. to the foot of the spike, 0.9 to the crest, and 1.2 msec. to the end; the time course of the second peak was 1.2 msec. to beginning, 1.45 to crest, and 2.0 to the end. Figure 2B was recorded slightly dorsal to Fig. 2A and had a second peak higher than the first. The peaks were at 0.95 and 1.3 msec., and there was a break on the descending limb at 1.8 msec.

The variation in time from stimulus artifact to the first break in the base

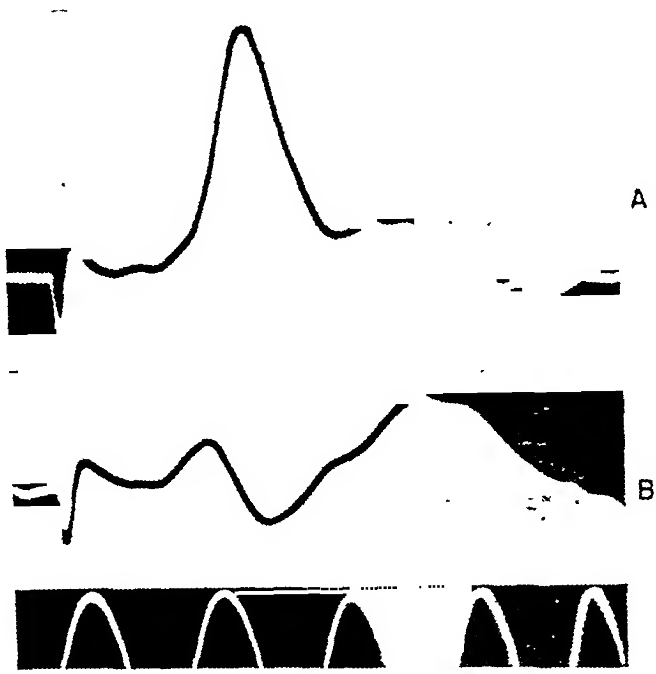

FIG. 4. Frontal nerve stimulated; other details as for Fig. 1.

line varied with conduction distance. The difficulty of measuring conduction distance along the course of the trigeminal nerve makes calculations of conduction rates, at best, close approximations. In seven determinations the average time for beginning of the wave at the entrance of the trigeminal fibers into the pons was 0.67 msec., whereas in eleven determinations the average time at the level of the upper cervical cord was 1.18 msec. The conduction distance between these points was about 25 mm., so that the fastest fibers conducted almost 50 m. per sec. within the brain stem.

The second wave of the action potential began 1.8 to 2.2 msec. after the stimulus. The magnitude and the time course of this part of the action potential were more variable and susceptible to change than was that of the first wave. At any one point, this second wave was constant for given experimental conditions but could be varied considerably or even abolished by

increased frequency of stimulation or oxygen deprivation. This wave varied, just as did the first, in the number of potential peaks. Figure 3A, B, and C are records from experiment 17 showing the changes in form of the second wave with changes in electrode position. As many as four peaks have been seen on the second wave, but with the larger number of peaks the intervals between crests tend to become shorter. At other times a low broad second wave, lasting to 4 or 5 msec. after the stimulus, was evident as in Fig. 3D.

The second wave, that part of the action potential beginning 2 msec. after the stimulus, is very likely due to activity in second order neurones. The wave was small when the recording needle was one millimeter or so dorsal or

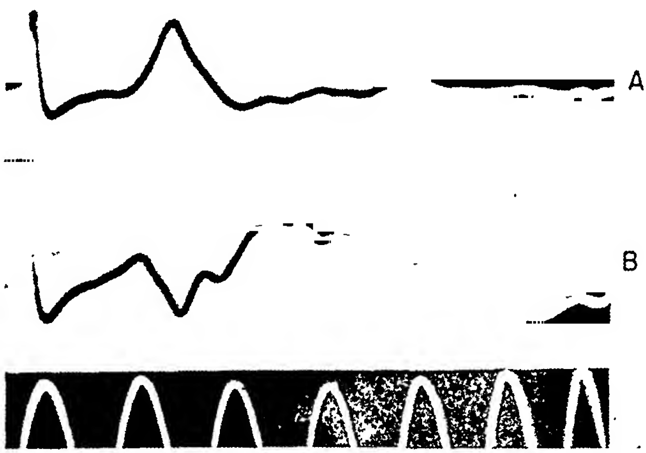

Fig. 5. Frontal nerve stimulated; other details as for Fig. 1.

lateral to the nucleus of the spinal tract. As the needle is moved ventrally or medially, the second wave becomes higher and the first wave smaller. Since the spinal tract overlies the spinal nucleus dorsally as well as laterally, the effect of moving the electrode 2 mm. ventrally from the tract into the nucleus was shown in experiment 16, Fig. 4A and B. The peak of the first wave was inverted and the second wave became larger. Figure 5A from experiment 15, with the needle in the spinal tract, had a small first wave and a negligible second wave, but, as is shown in Fig. 5B, upon moving the electrode one mm. medially, the first wave was inverted and a second wave appeared. Inversion of the first wave does not occur necessarily but depends entirely upon recording conditions (see 26 and 2, for discussions of the relation of the wave form to electrode position in a volume conductor). When the frequency of stimulation was increased from one in 2 sec. to 4 or 5 per sec., a decrease occurred in the second wave, especially in its latter portions, and at frequencies of 40 to 50 per sec. the wave practically was abolished. That oxygen deprivation depressed the second wave is shown by Fig. 6. Respiration ceased between records 6A and 6B, and artificial respiration was employed between

records 6B and 6C. The second wave disappeared within a few seconds after cessation of respiration whereas the first wave was still present several minutes later. In records 6A and 6C, the peak of the first wave was at 1.15 msec., but in record 6B continued to increase until 1.35 msec. The location from which the second wave is best recorded, the decrease in magnitude and duration with slight increases in frequency of stimulation, the susceptibility to oxygen deprivation, and the fact that the crest of the second wave fell 0.5 to 1.5 msec. after the crest of the first are all consistent with the belief that the second wave is produced by second order neurones. At times, a series of 3 or 4 crests occurred on the second wave with an interval of 0.6 msec. from

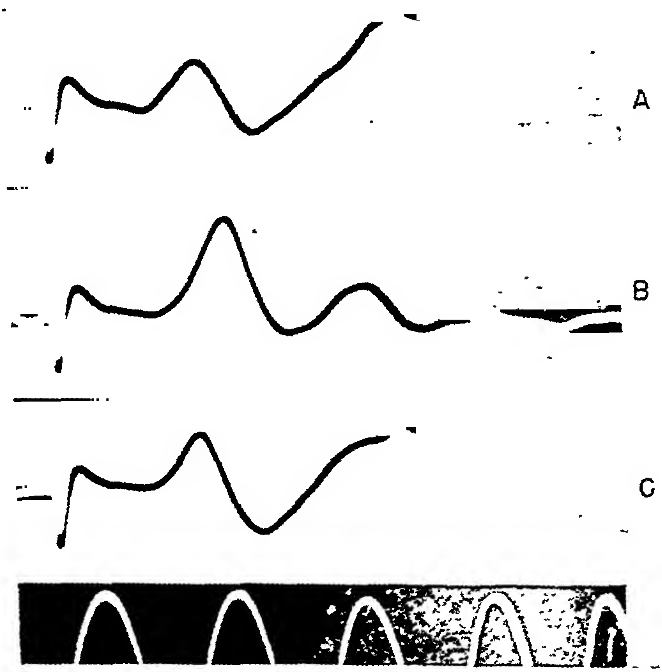

FIG. 6. Frontal nerve stimulated; other details as for Fig. 1.

one crest to the next. Renshaw (33) demonstrated that a single synapse may have a delay of 0.5 to 1.0 msec. but that delays appreciably longer than 1.0 msec. imply more than one synapse. It would be preferable, of course, to measure beginning of activity for each wave rather than the crest in each case but for many of the waves this is not possible. From the beginning of the first wave to the beginning of the second, the range of the delay was 0.8 to 1.1 msec. which is within the limits of a single synapse.

The potential waves elicited electrically were easier to trace than those from tactile stimulation but, even so, could not be followed significantly farther caudally. Figure 7 from experiment 16 illustrates the delineation of the caudal extent of the root. The frontal nerve was stimulated and records

were taken successively: Fig. 7A, with the needle in the dorso-lateral fasciculus of the caudal third of the first cervical segment, shows practically no activity; in 7B, one mm. rostrally, a small but definite first wave appears and shows that the caudal fibers of the spinal tract now are being contacted; and in 7C, one mm. farther rostrally, the presence of a large first wave shows that the recording electrode is now in contact with still more fibers. Similar observations were made for the caudal extent of the maxillary and mandibular fibers but at correspondingly higher levels.

Figures 8 to 13 are projection drawings from the brain stems of cats 6 and 10 and show diagrammatically the cross sectional relationships of the trigeminal divisions in the spinal root at different levels. Figure 14 is a diagram of the dorsal aspect of the pons, medulla, and cervical cord showing the course and caudal extent of three divisions.

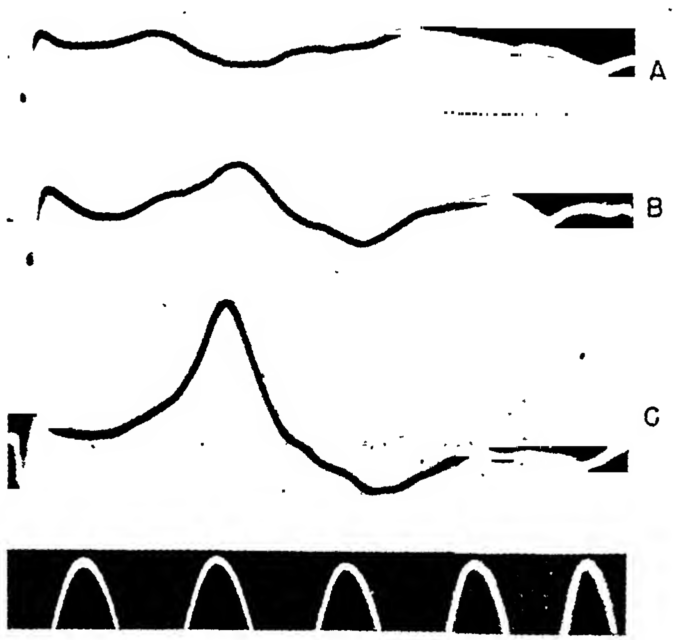

FIG. 7. Frontal nerve stimulated; other details as for Fig. 1.

DISCUSSION

Section of the spinal tract of the trigeminal nerve was carried out experimentally many years before being introduced as a surgical procedure for alleviation of facial pain. Laborde (25), in 1877, cut the tract in rabbits and dogs in order to compare the results with those obtained by earlier workers who sectioned the divisions of V or its sensory root. Observations were made chiefly on the eye, and loss of sensation and trophic changes were described. Sherrington (39), Ferrier (9), Biedl (1), and Van Gehuchten (12) were among others who experimentally sectioned the tract.

Ranson (32), in a paper read before the New York Neurological Society, presented his views on the fibers types in the spinal tract. In the discussion of Ranson's paper, Kuntz (p. 1140) and Elsberg (p. 1142) each suggested the possibility of cutting the tract for neuralgic pain. The main paper and dis-

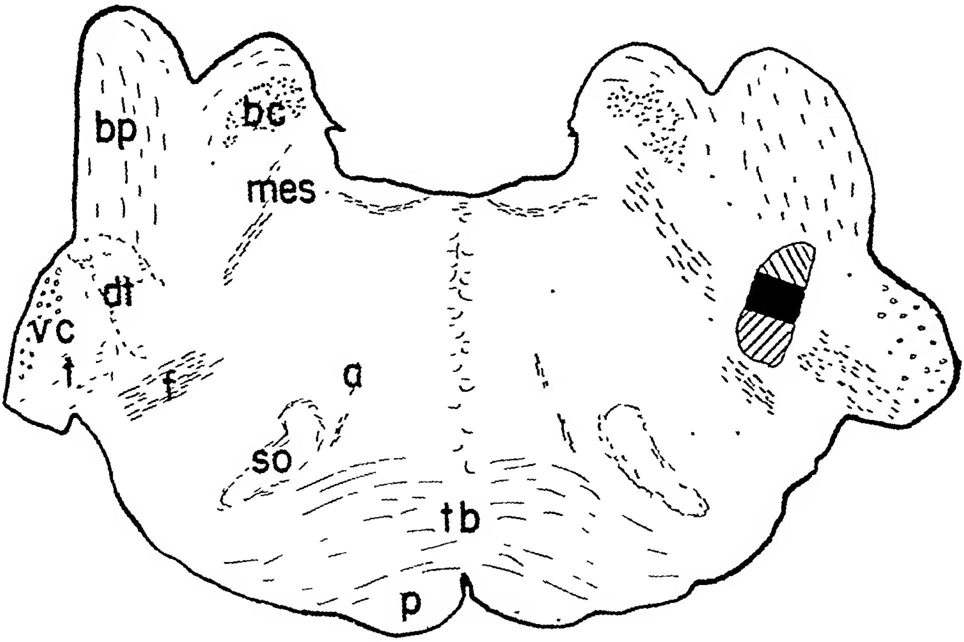

FIG. 8. Projection drawing of cross section of brain stem of cat 6. The positions of the fibers of the three divisions are shown diagrammatically on the right side; the dorsal cross-hatched area represents the mandibular fibers, the black area represents the maxillary fibers and the ventral cross-hatched area the ophthalmic fibers. This section is at a level just caudal to the entrance of the fibers into the pons. Abbreviations for all figures: a abducens fibers; AA area acoustica; AC ala cinera; AM anterior medullary velum; anc accessory cuneate nucleus; BC brachium conjunctivum; bp brachium pontis; Cl first cervical segment; CN cuneate tubercle; CP cerebellar peduncle; dp decussation of the pyramid; drcl dorsal root of the first cervical segment; dt descending or spinal tract of trigeminal nerve; dvr descending vestibular root; f facial fibers; fc fasciculus cuneatus; fg fasciculus gracilis; gf genu of facial nerve; GN gracile tubercle; HT hypoglossal trigone; hy hypoglossal fibers; IC inferior colliculus; io inferior olive; mes mesencephalic root of trigeminal nerve; nc nucleus cuneatus; ndt nucleus of the descending tract; ng nucleus gracilis; nhy hypoglossal nucleus; p pyramid; rb restiform body; so superior olive; svn superior vestibular nucleus; t trigeminal nerve; tb trapezoid body; TC tuberculum cinereum; ts tractus solitarius; vcn ventral cochlear nucleus; V1 ophthalmic division of the trigeminal nerve; V2 maxillary division of the trigeminal nerve; V3 mandibular division of the trigeminal nerve; VII facial nerve; VIII acoustic nerve.

cussion centered on the fact that here was an anatomical separation of functional fiber types and the possibility that pain could be abolished without loss of touch. Sjöqvist (39a) was unaware of the above discussion at the time he decided that section of the spinal tract was a feasible and worthwhile operation for relief of certain types of facial pain. In a monograph later the same year, Sjöqvist (40) referred to Ranson's paper and discussed

in detail the rationale of the operation which now bears Sjöqvist's name. Additional cases in which trigeminal tractotomy has been utilized clinically

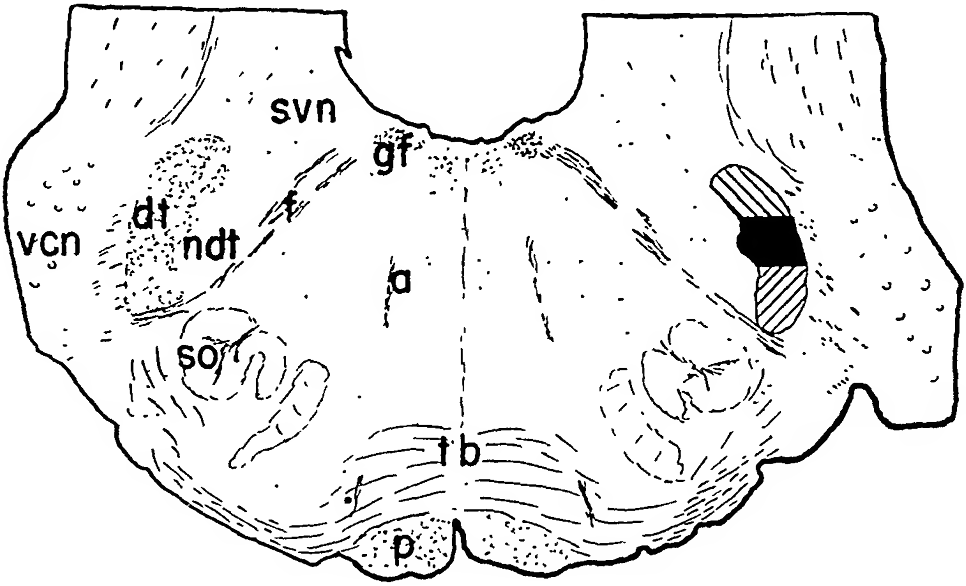

FIG. 9. Drawing of a section of the brain stem of cat 6 at the level of the facial nerve.

have been described extensively (15, 16, 17, 18, 22, 23, 29, 34, 35, 36, 41, 42). Using a similar approach, Schwartz and O'Leary (37) sectioned the spino-

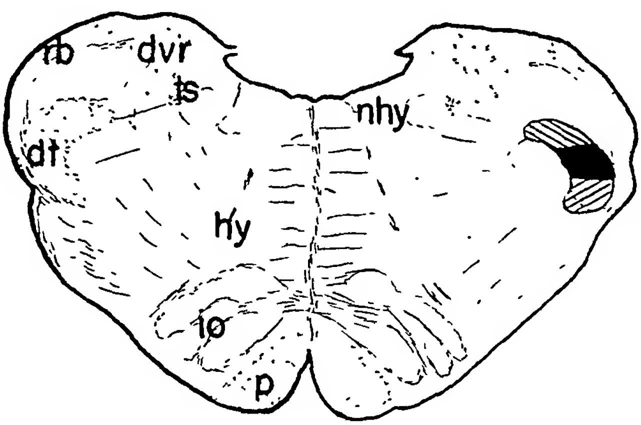

FIG. 10. Drawing of a section of the brain stem of cat 6 at the level of the hypoglossal nerve.

thalamic tract and part of the spinal trigeminal tract for intractable pain.

The strongest point in favor of tractotomy over root section by the Spiller-Frazier or Dandy methods is that touch grossly is preserved and

therefore there is less likelihood of subsequent trauma to the eye or other facial structures. Many investigators (5, 14, 21, 22, 42, 47, 48, 49, 50, 60), claim that the spinal nucleus receives only fibers of pain and temperature or state that there is no loss of tactile sensibility from lesions of the tract. Some

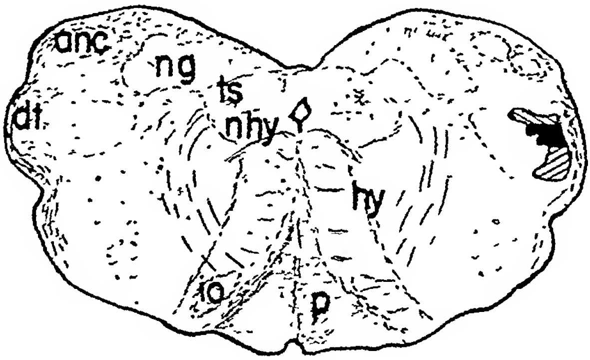

FIG. 11. Drawing of a section of the brain stem of cat 6 just caudal to the obex.

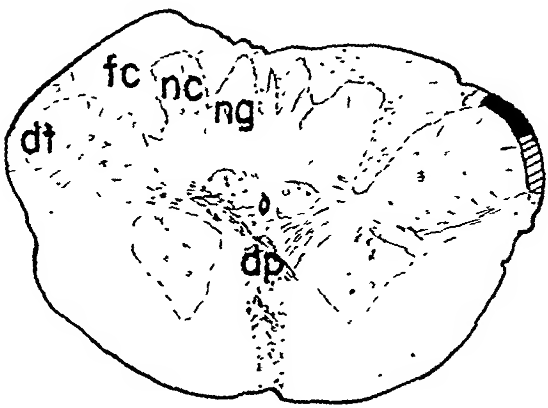

FIG. 12. Drawing of a section of the brain stem of cat 6 at the junction of the medulla and cord.

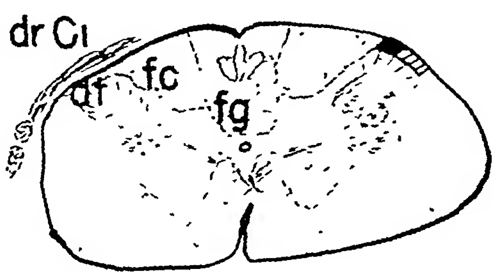

FIG. 13. Drawing of a section of the brain stem of cat 10 at the level of the rostral part of the first cervical segment.

workers very emphatically insist that touch impulses do not go to the spinal nucleus. As an example, Spiller (47), states, "In several papers, the first of which appeared in 1908, I showed that the spinal root of the trigeminal nerve contains pain and temperature fibers and does not contain fibers of tactile sensation."

However, careful clinical studies of patients subjected to trigeminal

tractotomy reveal that the spinal tract and nucleus also may mediate touch. Van Valkenburg (55) believed that touch is received by the spinal nucleus. Anatomically, the bifurcation of many trigeminal fibers as they enter the pons permits impulses to go both to the chief sensory nucleus and to the spinal nucleus. Windle (58) discussed this problem of the bifurcating fibers and decided that touch impulses travel both routes. Ranson (32) also pointed out the clinical implications of this anatomical arrangement. In the discussion (p. 1144) he stated, "I believe that these descending branches of the coarse fibers carry tactile impulses, and I believe that touch is mediated not only by the main sensory but also by the spinal nucleus, and therefore that when there is a lesion of the spinal fifth tract there may be some dulling of tactile sensibility. I should say that pain and temperature are mediated exclusively through the spinal fifth nucleus and touch through both the main sensory and the spinal fifth nuclei." This view has been substantiated fully by careful studies with von Frey hairs of patients with trigeminal tractotomy. Such studies have been carried out by Walker (56), Groff and his collaborators (15, 18), and Grant and Weinberger (16, 17). Sjöqvist (40), Rowbotham (34), and Olivecrona (29) also mention that, following tractotomy, the sensation of touch on the operated side is not normal. It thus seems that much of the confusion in the literature has resulted from the fact that many workers have tested for preservation of touch by stroking the face with cotton-wool but have not carried out the crucial tests with graded hairs as have the recent workers mentioned above. Such tests invariably reveal a measurable, though not necessarily disturbing, loss of tactile sensibility. Even though touch fibers do pass into the spinal tract it is likely that most of them are collaterals from fibers passing to the chief sensory nucleus. Therefore the statement

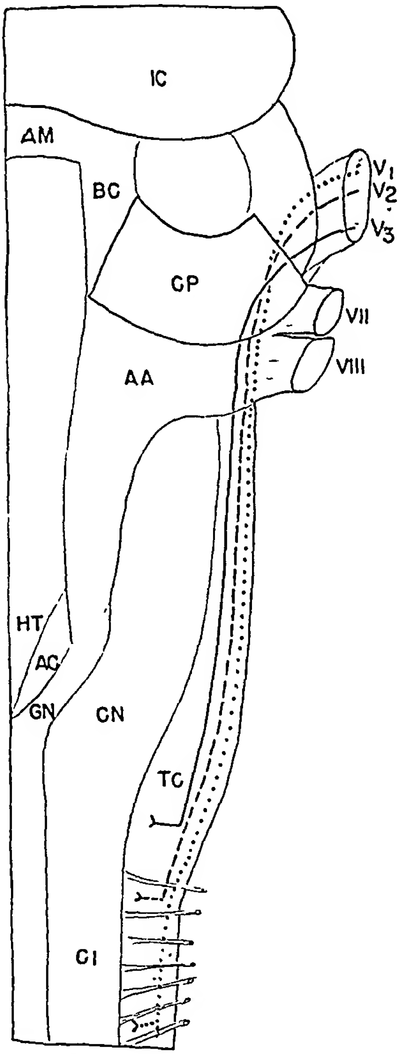

FIG. 14. A diagram of the dorsal surface of the pons, medulla, and upper cervical cord showing the course and level of termination of the fibers of the three trigeminal divisions.

of Grant and Weinberger (16) that, "Touch sensation for all practical purposes is preserved, and the patients are hardly aware that their faces have been made analgetic," undoubtedly is correct.

The experiments reported here show that in the cat a large number of tactile impulses can be traced into the spinal tract as far as the lower medulla and first cervical segment. In the monkey, Sjöqvist (40) found medium and large fibers in the spinal tract, presumably for mediation of touch, which disappeared before reaching the inferior olive. Gerard (14) and Windle (59) described similar medium to large sized fibers in the spinal tract of the cat.

There has been rather general agreement on the dorso-ventral arrangement of the trigeminal fibers within the spinal tract. Although many workers had sectioned successfully the sensory root in experimental animals and traced the central degeneration, Bregman (4) was the first to follow the degeneration centrally after partial section of the root. Upon cutting the sensory root in rabbits in such a manner that the corneal reflex was abolished, degeneration was found in the medial part of the sensory root and in the ventral part of the spinal tract. Sections of the root preserving the corneal reflex left the ventral part of the tract intact and degeneration was confined to the lateral part of the root and dorsal part of the tract. These findings were supported by the work of Wallenberg (57), cat and rabbit, by Bochenek (3), rabbit, Spiller and Frazier (46), dog, (11, for discussion) Van Valkenburg (54), various animals, and by Davis and Haven (7), cat. Without exception, these earlier workers found the fibers from the ophthalmic division in the ventral part of the spinal tract, the maxillary fibers in an intermediate position, and the mandibular fibers placed dorsally.

Clinical evidence supports the above experimental findings (15). Olivecrona (29), in describing his procedure, states, "If the incision has been placed correctly the pain felt at the moment of incision is referred to the entire trigeminal level. If pain is felt only in the first division, the incision should be extended a little more in the dorsal direction, since the fibers belonging to the third division lie in the dorsal part of the tract. Conversely, if pain is felt only in the third division, extension in the ventral direction is indicated." The only recent clinical report suggesting any arrangement other than that indicated is by Schwartz and O'Leary (37), who described loss of tactile sensibility over the mandibular distribution following damage to the ventrolateral part of the spinal tract in a single human case.

In the experiments here reported, there is direct physiological confirmation that in the spinal tract mandibular fibers are placed dorsally and ophthalmic fibers ventrally. Impulses from the ophthalmic distribution could be traced into the ventral part of the tract. The results indicate that there is little intermixture of the fibers of the three divisions within the spinal root. Pitts (30) used the spinal tract of the trigeminal to test localization of bipolar stimulating electrodes, and his Fig. 3 shows that, upon recording from the frontal nerve and stimulating centrally, the best response was obtained with the needle in the ventral third of the tract. Practically no response was

obtained from the frontal nerve with stimulation of the dorsal third of the tract. In a study of the afferent trigeminal pathways, McKinley and Magoun (27) also stated that the three divisions are laminated in inverse dorso-ventral order.

The caudal extent of each of the divisions has been a source of much disagreement, although uniformity of opinion has existed regarding the caudal terminations of the tract as a whole. The present experiments indicate that there are enough fibers from the ophthalmic division in the tract at the level of the caudal third of the first cervical segment to produce measurable potentials. Levels reported by others as the caudal extent of the spinal tract are: for the cat, Biedl (1), C2, Wallenberg (57), C1, Kljatschkin (24), C2, and Davis and Haven (7), C1; for the rabbit, Bregman (4), upper cervical cord, Laborde (25), the point of the calamus, Van Gehuchten (12, 13), lower C2, and Bochenek (3), at least C1; for the monkey, Tooth (53), upper C2, Sherrington (39), midway between C2 and C3, Ferrier and Turner (10), C2, and Ferrier (9), C2; for the guinea pig, Soukhanoff (44), upper cervical cord; for man, Obersteiner (28), C2, Poniatowsky (31), C2, Hun (21), upper C1, von Sölder (43), C2, Dejerine (8), C4, and Sjöqvist (39a), at least to C1. The fact that several species do not have a first cervical sensory root and that the sensory field of the second cervical nerve is contiguous with the trigeminal has led many workers to conclude that centrally the incoming sensory trigeminal neurones should be expected to reach the level of the incoming second cervical dorsal root. From the levels given above, it is evident that this is true. The description by Davis and Haven is typical of the degeneration studies in that, as the tract was followed caudally after section of the ophthalmic fibers, degeneration included an increasingly greater part of the ventral portion of the tract until, in the lowest sections, almost the entire tract was involved. The decrease in mandibular and relative increase in ophthalmic fibers caudally is indicated in Fig. 11, 12, and 13.

The results obtained here concerning the differential endings of the three divisions are consistent with the view most widely accepted at the present time. The levels were the lower part of C1 for the ophthalmic, the middle of C1 for the maxillary, and the junction of the medulla and C1 for the mandibular division. The conclusion that the ophthalmic division reaches farthest caudally was reached by many investigators (4, 5, 7, 29, 45, 50, 51, 54, 57). The alternative view that the distribution of fibers in the spinal tract is reflected peripherally as concentric rings converging on the bucco-nasal openings has been presented in varying forms by others (8, 43, 60). This view, which has been rejected by the majority of the workers on this subject, is discussed in full by Smyth (42) and Stopford (49). Since the spinal nucleus of the fifth nerve extends rostrally almost to the level of entrance of the trigeminal fibers and, since collaterals from the tract turn into the nucleus in abundance in its rostral part, it would be logical to suppose that section of the tract for relief of facial pain should be carried out as high as possible. The highest level that was considered safe by Sjöqvist (40) was the level of the

lowest vagal rootlet, at about the junction of the middle and caudal thirds of the inferior olive. Section at this level resulted in analgesia over all divisions peripherally but occasionally damaged the vagus nerve and restiform body. Grant and Weinberger (16), in later operations, decided that it was unnecessary to make the section so high, having found analgesia quite complete by making the incision 4 to 5 mm. below the obex and 2 mm. below the olive. This is said to be 12 to 14 mm. caudal to the point recommended by Sjöqvist. In one case, a section 8 mm. below the obex gave total and lasting anesthesia in all three divisions. Olivecrona (29) also decided, on the basis of several operations, that the Sjöqvist level was too far rostral and therefore sectioned at the level of the caudal pole of the olive, 2 to 3 mm. caudal to the level originally recommended. Olivecrona found that analgesia was just as complete when the trigeminal tract was cut at the lower end of the fourth ventricle as when it was divided 3 to 5 mm. higher. He concluded that comparatively few fibers leave the spinal nucleus to enter the secondary trigeminal pathway above the level of the lower end of the fourth ventricle. The many morphological descriptions of the spinal tract agree that the cross sectional area decreases slowly to the level of the exit of the hypoglossal nerve, decreases at a more rapid rate to the level of the decussation of the pyramids, and then quite rapidly as the cervical cord is reached. Sjöqvist's figures for the monkey show that the area at the level of the superior olive is 2 sq. mm., at the level of the entrance of the acoustic nerve, 2 sq. mm., at the caudal end of the inferior olive, 1.5 sq. mm., and at the pyramidal decussation, 1.5 sq. mm. Although it is not surprising that a high percentage of fibers in the spinal tract reaches as far as the lower medulla, it is interesting that the numerous collaterals given off at higher levels are not better able to carry on function in the cases operated for facial pain. In the preliminary report of McKinley and Magoun (27) it is stated that the axons of the second order pass diffusely from the main sensory and the spinal fifth nuclei, a statement made on the basis of oscillographic recording and one which would be expected from the anatomical arrangement.

In a review of all cases of Gasserian ganglionectomy up to 1896, Tiffany (52) stated that, "the history of operative measures for the relief of facial neuralgia is a history of operations on nerves at first peripheral, advancing slowly centrally." Since that time, the operative approach has progressed to the root section carried out by Spiller and Frazier (46), and to trigeminal tractotomy carried out by Sjöqvist. Serra and Neri (38) have carried out an operation which they believe destroys the first part of the "ascending trigemello-thalamic path." Reviews of trends in surgical procedures have been presented by various authors (6, 7, 13, 20, 40). Most recent papers, in discussing tractotomy, state that the procedure has many advantages over root section but, because of a few disadvantages, is not likely to replace the Spiller-Frazier operation. Since the approach is the same as that for the Dandy operation for root section, and is somewhat simpler, tractotomy may be the method of choice where the occipital approach is used. Even though

tactile sensibility is somewhat diminished by tractotomy, residual tactile sensation is so nearly normal that most patients are not aware of any post-operative difference.

SUMMARY

By means of the oscillograph, tactile impulses were traced into the spinal tract of the fifth nerve of the cat. Impulses originating in the ophthalmic division of the trigeminal were recorded as far as the caudal part of the first cervical segment, those from the maxillary area as far as the rostral part of the first cervical segment, and those from the mandibular area were followed to the junction of the medulla and cord.

Synchronous action potentials evoked electrically from a large branch of each of the divisions of the fifth nerve were recorded from the spinal tract and traced to approximately the same levels as given above. These potentials were easier to trace than the random tactile responses but could not be detected an appreciable distance below the levels mentioned. Physiological evidence is presented to support previous anatomical findings regarding the relative position of the three trigeminal divisions within the spinal tract. The ophthalmic fibers assume a ventral position, the mandibular fibers a dorsal position, and the maxillary fibers an intermediate position. Each division remains relatively independent throughout its course.

The action potential evoked electrically consisted of one or two waves each of which had one or more crests. The first wave was complete within 2 msec. and very likely resulted from activity in first order neurones. The second wave started at about 2 msec. lasted to as long as 4 or 5 msec., and evidenced activity in second or higher order neurones.

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EFFECTS OF INTENSITY AND WAVE LENGTH ON DRIVING CORTICAL ACTIVITY IN MONKEYS

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INTRODUCTION

IN A PREVIOUS paper we (3) have reported a driving effect upon the electroencephalogram (E.E.G.) of the unanesthetized monkey produced by intermittent photic stimulation of the retinae at various frequencies by flashes of relatively white or neutral light. It was found to be present on an average of 55 per cent of the time for the entire group and ranged from 28 to 78 per cent in individual monkeys when flashes of 120 footcandles were employed.

It is clear from the fact that we obtained these results without anesthesia, that coincidental intrinsic and extrinsic environmental factors did not greatly disturb the monkey or significantly interfere with its cortical activity. In fact the responsiveness of the animal has augmented our hope that the driving effect may be used as an indicator of cortical reactivity even in man. In order to ascertain the range of conditions under which the driving effect may safely be employed as an indicator of cortical reactivity, it is necessary to determine its dependency upon such factors as intensity of flash, wave length of flash, flash frequency and light dark ratio. Quantitative evidence is presented for the first two of these variables in the present report.

METHOD

Experiments were carried out on eight adult monkeys (*Macaca mulatta*). The procedure and apparatus‡ for securing simultaneous electroencephalograms from both occipital regions and a record of flash intervals have been described by us elsewhere (3).

Binocular stimulation was carried out with the unanesthetized monkey in a darkened, electrically shielded cage. Light from a tungsten source was interrupted at various frequencies by means of an interposed episcotister driven by a DC motor controlled by a rheostat. A light-dark ratio of 1/1 was employed throughout. Two convex lenses and a plane mirror were employed to condense and direct the light from its source to the surface of either neutral or monochromatic filters which were placed individually into a filter holder located at a distance of 25 cm. from the eyes of the monkey. An optical image was thus formed in the plane of the filter with the light diverging from that point to the eyes of the animal. In each experiment both pupillary and accommodative reflexes were eliminated by instillation of 0.5 per cent solution of scopolamine hydrochloride into the conjunctival sacs of both eyes. Stimulation was never begun until mydriasis was complete. Blinking movements were eliminated by continuous use of lid retractors during stimulation; a bit was employed to prevent head movements.

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‡ The authors are indebted to Dr. Theodore Case for use of the electroencephalographic equipment and to his assistant, Mr. Samuel Adams, for aid in solving various technical problems which arose in connection with apparatus.

Results

Effect of intensity. In determining the effect of variations in intensity upon driving, the following sequence of intensities was employed with two monkeys: 2, 4, 6, 8, 10, 20, 40, 60, 80, 100 and 120 footcandles. Data on three additional monkeys were obtained at 4, 40, 80 and 120 footcandles. The sequences were not reversed due to the relative slowness of dark adaptation. For each intensity value, flash frequency was either raised or lowered through 20 steps, the range of variation being in every instance from 3 to 12.5 flashes per sec. Each flash frequency was employed for 15 seconds for each intensity (tape speed was 3 cm. per sec.). Quantitative analysis was made of all records. Driving was considered present in any frequency when five consecutive

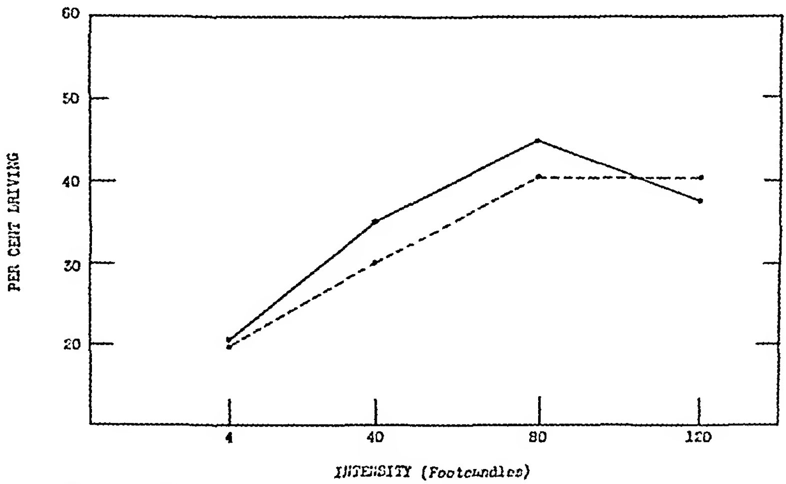

FIG. 1. Graph showing the average effectiveness in producing driving of the electroencephalogram in five monkeys by variations in intensity of intermittent flashes. Solid line—left occipital lead; broken line—right occipital lead.

waves of the E.E.G. coincided with the flash frequency. In many records the cortical frequencies followed the flash frequency throughout an entire period of photic stimulation at one intensity. The driving for each intensity was calculated in terms of the number of flash frequencies which produced driving out of the total of 20 frequencies employed. These values plotted against intensity are the basis for the curves in Fig. 1.

The results obtained for the two occipital regions are plotted separately. It will be noted that in general the two curves are quite similar and that both reach a maximum in the region of 80 footcandles. Flashes of neutral light at this intensity are about twice as effective in producing driving as four footcandle flashes. The explanation for this is not apparent. This intensity (80 footcandles) is well below the saturation point for discriminable brightness in man and presumably so for the monkey.

Effect of wave length. The effect of wave length upon driving was determined on seven monkeys (a total of 11 records) using the same optical set-up

as that employed for studying the influence of intensity except that monochromatic filters were substituted for neutral filters and the intensity was maintained at four footcandles throughout the experiment. All photometric determinations were made by means of a commercial model GM Photometer (GM Laboratories, Inc., Chicago, Illinois). This photometer consists of a galvanometer and a photo-electric cell with limiting filters to exclude all wave lengths but the visible spectrum for the human eye (sensitivity: 0.1 footcandle). Since the monkey and the human eye have been shown to have essentially similar spectral sensitivity curves (2) the photic stimuli were

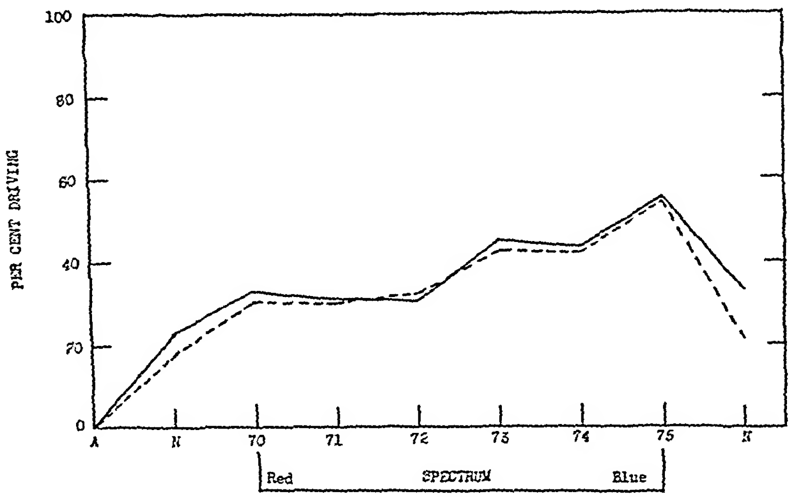

FIG. 2. Graph showing the average effectiveness in producing driving of the electrocencephalogram in seven monkeys (11 records) by variations in wave length of intermittent flashes. Solid line—left occipital lead; broken line—right occipital lead; A—auditory stimulus alone (control); N—neutral filter at four footcandles; 70–75—Wratten monochromatic filters equated at 4 footcandles.

equated for luminosity rather than for energy. The following sequence of stimulation was employed: an auditory control period, a neutral filter, Wratten monochromatic filters Nos. 70, 71, 72, 73, 74, 75, the neutral filter. The sequence of color filters and of flash frequencies was systematically alternated in the different experimental sessions for some monkeys. The per cent driving for each stimulus condition was calculated as for variations in intensity. These values plotted against their respective filters are the basis for the curves shown in Fig. 2. The transmission of each of the color filters employed is shown in the table given on the next page.

The relative effectiveness of various parts of the spectrum in producing driving is shown by the curves plotted in Fig. 2. The per cent driving for each occipital lead is plotted separately. Again it may be noted that the two curves are quite similar and that both reach a maximum in the blue region of the spectrum. In spite of considerable variation in the absolute percentage of driving for each occipital lead from one animal to another, it is apparent that

Table 1. Transmission of Wratten filters (1).

Filter no.	Range of transmission	Transmission maxima
70	635–700 mμ	700 mμ
71	600–700 mμ	640 mμ; 700 mμ
72	585–700 mμ	610 mμ; 700 mμ
73	575–700 mμ	585 mμ; 700 mμ
74	510–570 mμ	530 mμ
75	455–535 mμ	490 mμ

in general, the blue end of the spectrum tended to be more effective in producing driving than the red end. If we express this difference in terms of the extremes of our spectrum, blue filter No. 75 produced an average driving of 55 per cent as compared with red filter No. 70 which produced an average driving of 31 per cent, a difference which is quite reliable.

Relative driving effect of colored versus neutral light. The average per cent driving produced by all six of the colored filters was 38 per cent as compared with 23.5 per cent for the neutral filters. In other words, the relatively monochromatic light was approximately 1.6 times as effective in producing driving as neutral light of the same luminous energy.

Effect of temporal sequence on driving. Reversing the sequence of the monochromatic filters did not alter significantly the relative driving effectiveness of any of them. A slight general tendency for increased driving during the 45-minute experimental period is suggested, however, by the fact that neutral light was somewhat more effective on the average in producing driving at the end of the period than towards the beginning. The average of the two occipital leads gave 20 per cent driving in the second 5-minute interval and 27 per cent driving in the ninth 5-minute interval in which neutral light was used.

Summary

From an investigation of the effects of intensity and wave length on driving the cortical activity in monkey (*Macaca mulatta*) it was found that: (i) the greatest driving was obtained for variations in intensity with flashes of 80 footcandles. At this value flashes were approximately twice as effective in producing driving as flashes at 4 footcandles; (ii) monochromatic light is relatively more effective (1.6 times) in producing driving than neutral light of the same luminous energy; (iii) the blue region of the spectrum was considerably more effective in producing driving than the red region.

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REFLEX DISCHARGES IN BRANCHES OF THE CRURAL NERVE

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THIS PAPER describes reflex discharges into those branches of the crural (femoral) nerve which supply the quadriceps extensor and sartorius muscles. The discharges were evoked by stimulation of dorsal roots and were conditioned by the stimulation of other dorsal roots and nerves of the ipsilateral hind limb.

The discharges in the nerve to the quadriceps, in particular its vastus internus portion, were studied because of the function of this muscle as the principal effector organ of the knee-jerk (8). In view of the fact that the knee-jerk has been described as a reflex of brief central latency which is immediately inhibited by stimulation of nerves in the ipsilateral hind limb (9, 1), it was of interest to examine the activity of its component neurons in the light of the newer observations on central reflex times and spinal inhibition (6, 7, 3). In the cat most of the motoneurons innervating the vastus internus lie in the fifth and sixth lumbar (L5 and L6) segments of the spinal cord, and the excitatory afferent axons for the knee-jerk pass into the cord via the dorsal roots of these segments, particularly L6 (8). The well-known inhibition of the knee-jerk by stimulation of such a nerve as the ipsilateral peroneal or hamstring (9, 1) involves impulses in sensory fibers, many of which enter the cord over the L7 dorsal root.

The axial distribution within the cord of the motoneurons which supply the sartorius overlaps that of the quadriceps motoneurons, the sartorius pool being shifted a little in the cephalic direction (8), and the motor fibers to both muscles pass peripherally in the crural nerve. In contrast with the action of the vastus internus as an extensor of the leg, the principal role of the sartorius is to flex the thigh. It therefore seemed of interest to compare the reflex discharges into the nerves of these two muscles which have entirely different functions; but whose motoneurons are in topographical association.

METHODS

The experiments were performed on cats which had been narcotized with pentobarbital sodium ("Nembutal") or Dial (Ciba). The branches of the crural nerve which supply the vastus internus and sartorius muscles were cut distally and placed on recording electrodes for monophasic recording. The remaining branches of the crural nerve were severed, as were the obturator nerve and the iliopsoas muscle. After a laminectomy had been performed, the necessary dorsal roots were cut intradurally and placed on stimulating electrodes. The shocks delivered to the dorsal roots were usually of a strength two to three times the threshold for their most excitable fibers. The usual differential amplifier, oscillograph, and stimulating apparatus were used.

Results

1. *Difference between reflex discharges into motor nerves supplying vastus internus and sartorius muscles.* Under the conditions of the present experiments, the reflex discharges into motor branches supplying the sartorius differed considerably from those in branches to the vastus internus and other components of the quadriceps. Furthermore, the reflex discharges to the two muscles were conditioned in different ways by antecedent stimulation of the same groups of sensory axons. These differences, which existed despite the

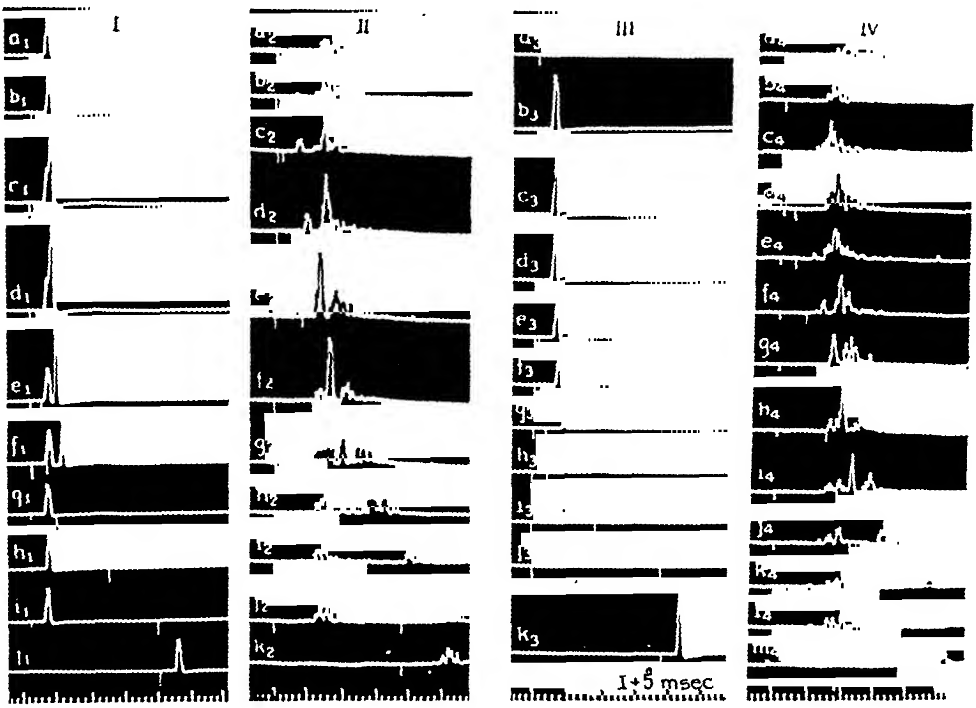

FIG. 1. Comparison of reflex discharges into the nerves to the vastus internus (columns I and III) and sartorius (columns II and IV) muscles. Columns I and II show the responses to one and two L6 dorsal root volleys. Columns III and IV show the responses to an L7 dorsal root volley (records *a*); an L6 volley (records *b*, *k* 3, *m* 4; and to an L7 volley plus an L6 volley (records *c* 3–*j* 3 and *c* 4–*l* 4). The amplification for the records of column III was *ca.* 1.7 times, for columns II and IV five times, that for column I.

overlapping axial distribution of the motoneurons supplying the two muscles (8), are illustrated by the records of Fig. 1. The discharges shown in columns I and III were recorded from the nerve to the vastus internus; those in columns II and IV from a branch to the sartorius. The motor discharges were initiated by L6 dorsal root stimulation and were conditioned by L6 dorsal root volleys (columns I and II) and L7 dorsal root volleys (columns III and IV).

A nearly synchronous discharge of brief central latency was evoked in

the vastus internus nerve by an L6 dorsal root volley (records *a* 1 and *j* 1). Records *b* 1 to *f* 1 reveal that an L6 volley facilitated the motoneurons in question to a second L6 volley for a brief period of only 2 to 3 msec. There followed a prolonged period of profound inhibition, the beginning of which is illustrated in records *g* 1 to *i* 1. In contrast, the reflex discharge set up in the nerve to the sartorius by a L6 dorsal root volley had a different nature (record *a* 2). It was characterized by impulses of relatively long central latency. In this preparation no impulses of short central reflex time appeared in response to a single L6 dorsal root volley (compare record *a* 2 with *a* 1). The responses to two L6 dorsal root shocks showed that the arrival of an L6 volley at the cord facilitated the sartorius motoneurons for *ca.* 10 msec.—considerably longer than it facilitated the vastus internus motoneurons. A prominent feature of the conditioned discharge was the appearance of impulses of short central latency. At longer shock intervals the discharges to the second L6 volley were inhibited (*cf.* records *i* 2 and *j* 2), but not as profoundly nor for as long as in the case of the vastus internus motoneuron discharge.

Further differences between the responses of vastus internus and sartorius motoneurons appeared when the reflex discharges to L6 volleys were conditioned by L7 dorsal root volleys (columns III and IV). Afferent impulses in the L7 dorsal root produced no conspicuous discharge of vastus internus motoneurons (record *a*3). The only patent effect on the discharge initiated by a subsequent L6 volley was inhibitory (records *d* 3 to *j* 3). The inhibition was profound and prolonged, and the latency of its onset very brief (cf. record *d* 3). On the other hand, an L7 dorsal root volley produced a discharge of sartorius motoneurons (record *a* 4). This discharge was similar to that produced by an L6 volley (records *b* 4, *m* 4, *a* 2 and *k* 2). Furthermore, an L7 volley facilitated the response to a subsequent L6 volley for a period of over 12 msec. At longer L7-to-L6 shock intervals, the discharge produced by the L6 volley was inhibited.

Figure 2 shows the results of two additional experiments in which the discharges into the sartorius nerve were examined. In the experiment of column I the discharge of record b_1 was produced by an L7 dorsal root volley, of records a_1 and i_1 by an L6 dorsal root volley, and of records c_1 to h_1 by an L7 plus an L6 volley. The results were similar to those shown in column IV of Fig. 1—both an L6 and an L7 volley produced relatively prolonged discharges of long central latency, and the discharges produced an L6 volley were facilitated when the L6 volley followed the L7 volley by 0–10 ± msec. An additional fact was revealed in the experiment from which the records of column II were taken. In this experiment the testing shock stimulated the fibers of the L5 as well as the L6 dorsal root. The discharge evoked in the sartorius nerve by their combined stimulation (record *b*2) showed in addition to discharges of relatively long central latency, a discharge of short central reflex time—about 1.0^- msec. This discharge, like the discharges of short central latency into the nerve of the vastus internus,

was inhibited by an L7 dorsal root volley which preceded the L6 dorsal root volley at short intervals of less than 1.8 msec. (records *d* 2–*f* 2). Unlike the impulses in the vastus internus nerve, however, this sartorius motoneuron discharge of short central latency was clearly facilitated when the shock to the L7 dorsal root preceded the L6 shock by 2 to *ca.* 12 msec., as is shown by record *g* 2 for a shock interval of 2.2 msec.

These characteristics of the discharges in the nerves to the vastus internus and sartorius appeared consistently in a series of preparations. The

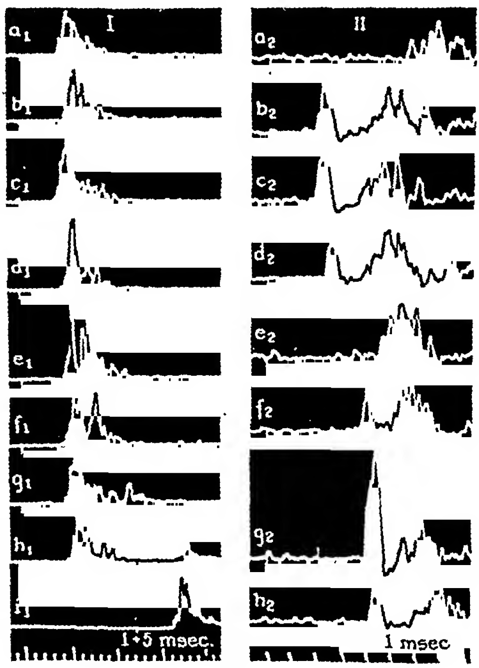

FIG. 2. Reflex discharges initiated in the nerve to the sartorius by ipsilateral dorsal root volleys; records *b* 1 and *a* 2 by an L7 dorsal root volley; records *a* 1 and *i* 1 by an L6 dorsal root volley; records *c* 1–*h* 1 by an L7 followed by an L6 dorsal root volley; records *b* 2 and *h* 2 by a volley in L5 and L6 dorsal roots; records *c* 2–*g* 2 by an L7 volley followed by an L5 plus L6 volley.

constancy of the discharges stands in contrast with the relatively variable reflexes which are recorded from ventral roots in comparable preparations. An explanation for this difference lies in the fact that a ventral root contains axons passing to several different muscles. A mere quantitative difference between the relative sizes of the discharges passing to such a muscle as the sartorius on the one hand, and to such a one as the vastus internus on the other, would explain the variability which is experimentally observed in the ventral root discharges from a series of similar preparations.

2. *Excitation and inhibition of motoneurons supplying the quadriceps (vastus internus).* The data of Fig. 3–5 have been taken from typical experiments to demonstrate (i) the central reflex times for discharges evoked in the nerve to the vastus internus by stimulation of the L6 (also L5) dorsal root, and (ii) the time course of the inhibition of this discharge as a consequence of conditioning volleys in the L7 dorsal root.

In the experiment from which the records of Fig. 3 have been taken, a single L6 dorsal root volley evoked a sizable reflex discharge into the nerve of the vastus internus (oscillogram *b*). The discharge represented a slightly dispersed volley of impulses, the central latencies for which were determined by calculation of the times for conduction in afferent and efferent fibers. Record *c* shows the potential changes which were set up as a result of the application of a shock to the L6 dorsal root and recorded at an electrode placed on the

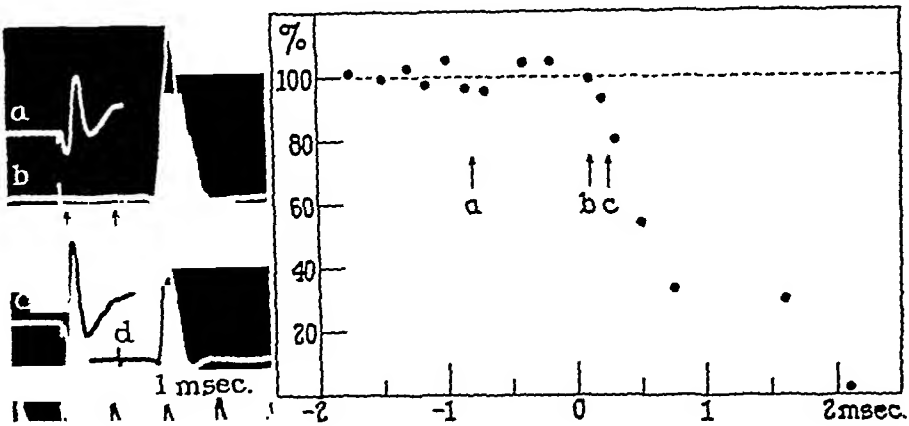

FIG. 3. Inhibition of the reflex discharge in the nerve of the vastus internus by an L7 dorsal root volley. The testing stimulus which evoked the reflex was an L6 dorsal root volley.

$$\text{Ordinates: } \frac{\text{Average height of conditioned reflex}}{\text{Average height of unconditioned reflex}} \times 100.$$

Abscissae: Interval at which the shock to the L6 dorsal root followed the shock to the L7 dorsal root. The significance of the arrows is described in the text. Oscillograms: *a*, the potential changes which were evoked at the dorsal surface of the L6 segment of the cord as a consequence of the delivery of a shock to the L7 dorsal root; *c*, same, due to the L6 dorsal root volley; *b*, the unconditioned reflex discharge set up in the nerve to the vastus internus by delivery of a shock to the L6 dorsal root; *d*, response at the recording electrodes on the nerve to the vastus internus due to direct electrical stimulation of the motoneurons at the L6 segment of the cord.

dorsum of the cord at L6. An indifferently placed electrode completed the recording circuit. Oscillogram *a* is a similar record obtained in response to stimulation of the L7 dorsal root. Conduction time in the dorsal roots was measured as the interval between the stimulus escape and the moment at which the oscillograph spot started to rise to the base-line from the first positive (downward) trough of the primary spike of the cord potential (2). An electrical shock was then delivered directly to the motoneurons of the L6 ventral horn and the resulting motor discharge recorded at the electrodes on the nerve to the vastus internus (record *d*). The shock-response interval for such an *m* wave (4, 5) has been shown to be a valid measure for conduction time in the motor fibers of a reflex arc (6, p. 377).

Utilizing these corrections for conduction times in the L6 dorsal root and in the motor fibers, it is seen that the afferent volley arrived at the L6 segment of the cord at the time of the first arrow (record *b*) and that the first reflex impulses were initiated in the motor axons at the time of the second arrow. The interval between the arrows—0.95 msec.—represented the minimal central latency for the reflex discharges. Minimal central reflex times of 0.9 to 1.6 msec. were obtained in other experiments. The shortest times presumably represented discharges in two-neuron reflex arcs.

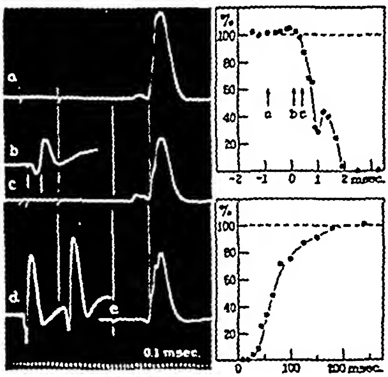

FIG. 4. Same as Fig. 3, except that the tested reflex was evoked by the second of two L6 dorsal root volleys. Oscillograms: *a*, unconditioned testing response; *c*, conditioned testing response; *b*, potential changes at the dorsum of the cord at the L6 segment due to a conditioning (L7) dorsal root volley; *d*, same, due to the two L6 dorsal root volleys; *e*, "*m* wave" at the recording electrodes on the nerve to the vastus internus due to direct electrical stimulation of the motoneurons at the cord.

The graph of Fig. 3[1] demonstrates the effects of a conditioning L7 dorsal root volley on the reflex discharge shown in oscillogram *b*. The conditioning was inhibitory and the latency of the inhibition was brief; indeed, it was already evident at a conditioning-shock to testing-shock interval of 0.2 msec. Making corrections for the conduction of impulses in the L6 and L7 dorsal roots and in the motor fibers to the vastus internus, it turns out that the arrow *a* marks the shock interval (L7 following L6 by 0.8 msec.) at which the tested impulses were set up in the motor axons just at the moment when the conditioning L7 dorsal root volley arrived at the cord. For points on the graph to the left of arrow *a*, the conditioning impulses arrived at the cord after the tested reflex impulses had been set up and passed peripherally into the motor axons; therefore a conditioning effect could scarcely have been expected. For points on the graph to the right of arrow *a*, the conditioning impulses arrived before the tested motoneurons fired. At the shock interval indicated by the arrow *b*, the conditioning and testing dorsal root

[1] In the graph of this and subsequent figures, the degree to which the tested discharge was altered as a result of the conditioning stimulation is indicated on the ordinate scale: and, as is customary, the abscissae represent the interval at which the testing shock followed the conditioning shock. The only unusual feature is that some of the graphs have been extended to the left of the zero shock interval to include negative values. A negative conditioning-shock to testing-shock interval merely means that the conditioning shock followed the testing shock. Because the tested motor impulses were initiated only after a latency which included both the conduction time in testing dorsal root fibers and the central reflex time, a conditioning shock could follow the testing shock and still precede the time of initiation of the tested motor impulses.

volleys arrived simultaneously at the L6 segment of the cord. The interval between arrows *a* and *b* therefore represents the central reflex time for the tested impulses. Only if the conditioning impulses arrived slightly earlier with respect to the time of initiation of the tested motor impulses (as indicated by arrow *c*), was the inhibition clearly established. Therefore the interval between arrows *a* and *c* represents the minimal time by which the arrival at the cord of the conditioning volley had to precede the firing of the tested motoneurons in order that inhibition could be observed. This interval

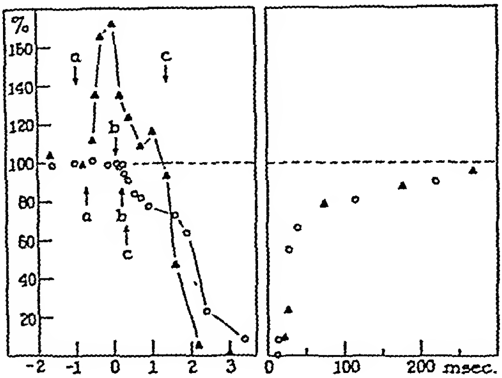

FIG. 5. Conditioning curves as in Figs. 3 and 4. The tested reflex was evoked in the nerve to the vastus internus by two L5 dorsal root volleys. ▲—conditioning by an L6 dorsal root volley; O—by an L7 dorsal root volley. The significance of the arrows is described in the text.

may be defined as the *central inhibitory time* for the effects of the conditioning volley on the tested motoneurons. In this experiment it amounted to 1.0 to 1.1 msec. This value is about the same, or slightly longer than, that reported for the previously studied cases of the so-called direct inhibitory action of centripetal sensory and motor volleys (3, 7).

The total time course of the inhibitory action is illustrated in Fig. 4. In the preparation from which the data of this figure were taken, a single L6 dorsal root volley produced only a small reflex discharge with a minimal central reflex time of 1.6 msec. The response to the second of two L6 dorsal root volleys which were spaced at 0.95 msec. was larger and had a minimal central latency of 1.0⁻ msec. (record *a*). This facilitated motoneuron discharge was first inhibited when conditioning impulses in the L7 dorsal root arrived at the L6 segment of the cord 1.2 msec. before the firing of the tested motoneurons. As the interval between the L7 and the testing L6 dorsal root volleys increased, the inhibition became complete, subsequently to disappear only when the tested motoneuron discharge followed the conditioning volley by nearly 200 msec. (0.2 sec.). In comparable experiments the duration of the period of inhibition was not shortened after the intravenous injection of sufficient curare to abolish all muscular contractions.

The similarities and differences between the effects of L6 and L7 dorsal root volleys on the vastus internus motoneurons may likewise be demonstrated by initiating a motoneuron discharge with an L5 dorsal root volley and conditioning with L6 and L7 volleys. In the experiment from which the

data in Fig. 5 were taken, the tested vastus internus motoneuron response was initiated by the second of two closely spaced L5 dorsal root volleys. The curve marked with closed triangles was the result of conditioning by an L6 dorsal root volley; that marked with open circles was due to conditioning by impulses in the fibers of the L7 dorsal root. The arrows have the same significance as in the preceding figures. Those above the 100 per cent line relate to the L6 conditioning; those below the line to L7 conditioning. Arrows *a* mark the conditioning-shock to testing-shock interval at which the tested motoneurons fired simultaneously with the arrival of the conditioning volley at the cord, at the axial level of the boundary between the L5 and L6 segments. Arrows *b* mark the interval at which the conditioning volley arrived at the cord simultaneously with the testing volley in the L5 dorsal root; and arrows *c*, the shock interval at which the inhibition due to the conditioning impulses was first apparent. The central reflex time for the tested motor impulses (the *a–b* interval) was about 1.0 msec. Facilitation was produced by an L6 dorsal root volley which arrived at the cord, at the level of the boundary between the L5 and L6 segments, 0.5 ±—2.3 ± msec. before the discharge of the tested motoneurons. When the conditioning impulses arrived earlier, inhibition was apparent; it lasted until the conditioning-shock to testing-shock interval exceeded 200 msec. An L7 dorsal root volley produced only inhibitory effects, except possibly when it preceded the tested motoneuron discharge by more than 200 msec. —a postinhibitory "rebound" has been noted in some experiments (*cf.* Fig. 7). The inhibition had a central latency (central inhibitory time) of 1.1—1.2 msec.; that is, a condition for the appearance of the inhibitory action was that the L7 impulses arrive at the cord 1.1—1.2 msec. before the firing of the tested motoneurons. The degree of inhibition became greater as the conditioning-shock to testing-shock interval became longer; at still longer shock intervals it declined along the same time course as the inhibition produced by an L6 dorsal root volley.

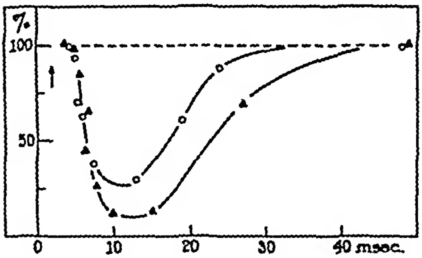

FIG. 6. Conditioning curves as in preceding figures. The tested reflex was evoked in a motor nerve to the quadriceps by two L6 dorsal root volleys. A volley in the sural nerve conditioned the response according to the curve marked (▲); a volley in a small branch to the biceps according to the curve marked (○). The arrow denotes the shock interval at which the conditioning (L7) and the testing (the second L6 dorsal root) volleys arrived simultaneously at the cord.

The inhibition of the vastus internus motoneuron discharge by the stimulation of such nerves of the hind leg as the hamstring or peroneal is similar to that produced by L7 dorsal root stimulation, provided that account is taken of the greater time required for the conduction of the centripetal volley.

Use of a conditiong volley in a small peripheral nerve, as well as of a submaximal alpha volley in a larger nerve or the L7 dorsal root, produced an inhibitory effect which had a longer latency and shorter duration than that produced by the larger volleys described above. The effect was also less profound; and because the inhibition of the testing response was not complete, it was possible to show that the inhibitory effect attained its maximal value when the conditioning impulses arrived at the cord 10–15 msec. before the discharge of the tested motoneurons. These features of the inhibitory process are shown in Fig. 6 and 7. In Fig. 6 the curve marked with open circles represents the inhibition which was produced by a volley in a small branch to a hamstring muscle. Stimulation of the sural nerve—a relatively small cutaneous nerve—produced the curve marked with closed triangles. It is seen that the stimulation of sensory fibers from cutaneous and deep parts of the hind limb had similar effects on the vastus internus motoneurons.

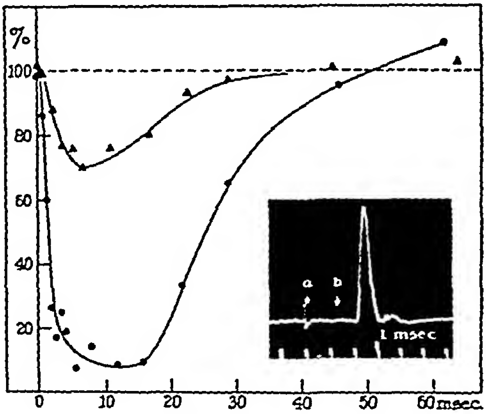

FIG. 7. Conditioning of vastus internus motoneuron discharge by impulses in sensory and motor fibers of the hamstring nerve. The inset shows the tested reflex discharge which was set up in the nerve to the vastus internus by a dorsal root volley in the L5 plus L6 dorsal root fibers. The coordinates are as in the preceding figures. The curve (●) shows the conditioning of the tested reflex by a volley in the hamstring nerve. The L7 and sacral dorsal roots were then cut. Since the L5 and L6 dorsal roots had been cut at the beginning of the experiment, the hamstring nerve was thereby deafferented. A centripetal volley in the hamstring nerve then conditioned the tested reflex as shown by the curve (▲).

Since the inhibitory effects of brief central latency have been demonstrated to be produced by centripetal volleys in motor fibers (7), it is of interest to differentiate between the effects of the sensory and the motor components of a centripetal hamstring volley up on the inhibition of vastus internus motoneuron discharges. The testing vastus internus motoneuron response shown in the inset of Fig. 7 was produced by the stimulation of the L5 plus L6 dorsal roots. As shown by the interval between the arrows, the central reflex time for the first impulses of this discharge was about 1.25 msec. The points shown as solid circles represent the conditioning effects of a volley of impulses in the alpha fibers of the hamstring nerve. It therefore represented the effects of impulses in both sensory and motor fibers. The dorsal roots of the L7 and sacral segments were then cut intradurally, leaving intact only the motor fibers of the hamstring nerve. Stimulation of the hamstring nerve then conditioned the tested vastus internus motoneuron discharge, as is shown by the points marked with solid triangles. The curve (●) therefore represents the combined effects of cen-

tripetal sensory *plus* motor impulses; the curve (▲) only the effect of motor impulses. Obviously the action of the centripetal motor impulses was a not negligible fraction of the effect of sensory plus motor impulses. Account must be taken of such actions of antidromic motor impulses in experiments in which mixed peripheral nerves are stimulated. This is especially true in view of the fact that the inhibition of quadriceps motoneuron discharges by hamstring motor volleys is not as great as the inhibition of the discharges of many groups of motoneurons by antidromic volleys in other motor axons—for instance, the inhibition of vastus internus motoneuron discharges by antidromic volleys in other motor branches to the quadriceps (7).

Discussion

The net result of an L7 dorsal root volley on the excitability of the vastus internus motoneurons, as tested in the present experiments, was a powerful inhibitory action of brief central latency and long duration. Yet it must not be supposed that stimulation of such a large and heterogeneous population of afferent fibers as comprise a dorsal root would result in the production of only inhibitory actions, direct and relayed through interneurons, on the tested motoneurons. Indeed, the conditioning curve of Fig. 4 suggests the presence of a very slight facilitation of the tested discharge previous to the onset of its inhibition; and at shock intervals of 1 to 2 msec. in the curves of Fig. 3, 4, and 5, there is a discontinuity which might be interpreted as evidence for a submerged facilitatory action of the conditioning volley. Thus one cannot state whether the observed minimal central inhibitory time of *ca.* one msec. would have been shorter had impulses in fibers responsible for an excitatory action on the tested motoneurons been excluded from the conditioning volley. At any rate, in the present experiments a conditioning (inhibitory) volley, which arrived at the cord *immediately* before the discharge of the tested motoneurons, produced no demonstrable inhibitory action. A condition for the appearance of a demonstrable inhibitory action was that the conditioning volley arrive at least one msec. before the firing of the tested motoneurons; that is, the inhibitory volley had to arrive at the cord approximately simultaneously with, or earlier than, the testing dorsal root volley which fired the tested motoneurons after a central reflex time of *ca.* one msec.

As has been pointed out (6, p. 381 *et seq.*), one cannot at present state whether central reflex times as long as one msec. pertain to 2-neuron arcs, or to 3-neuron arcs, or to both. In the present experiments the central reflex times for the tested motor impulses into the nerve of the vastus internus, and the central inhibitory times for the effect of the L7 dorsal root impulses upon them, have had durations of *ca.* one msec. and, in some experiments, even a little more. It has therefore seemed wise to be noncommittal as to whether or not the tested reflexes were strictly 2-neuron arc discharges, as well as to whether the inhibitory effect was necessarily mediated by the direct action of the dorsal root fibers on the tested motoneurons.

It must be emphasized that the results described in this paper may apply only to preparations such as those which were studied—that is, to cats under barbiturate anesthesia, with the stimulated dorsal roots and the examined motor nerves cut peripherally. The nature of the reflex discharges in the nerves of the vastus internus and sartorius might be different in other types of preparations, just as the character of the knee-jerk and the inhibitory action of a hamstring volley upon it differ greatly according to whether the preparation is decerebrated or spinal (1).

Summary

The sartorius and the vastus internus are two muscles of different function which are supplied by motor axons originating in the same and adjacent segments of the spinal cord and passing together peripherally in the crural nerve. In a series of cats under pentobarbital sodium anesthesia, reflex discharges were evoked in the motor nerves to each of these muscles by the stimulation of dorsal roots. The discharges had a constancy from preparation to preparation, which stands in contrast with the variability observed in ventral root discharges similarly evoked in comparable preparations.

In spite of the topographical association of the two groups of motoneurons, the reflex discharges in the motor branches supplying the sartorius differed considerably from those in branches to the vastus internus and other components of the quadriceps. Furthermore, the reflex discharges to the two muscles were conditioned in different ways by antecedent stimulation of the same groups of sensory axons. An L6 dorsal root volley evoked in the nerve to the vastus internus a nearly synchronous discharge having a central latency of only 0.9 to 1.6 msec. It evoked in the nerve to the sartorius a dispersed discharge of relatively long central latency. An L7 dorsal root volley produced no conspicuous discharge of vastus internus motoneurons. Its only effect on the discharge produced by an L6 volley was inhibitory. The profound inhibitory effect had a duration as long as several hundred msec. The latency of its onset was brief; it was already present when the conditioning volley arrived at the L6 segment of the cord only 1.0 to 1.1 msec. before the firing of the tested motoneurons. In contrast, an L7 volley produced in the branches to the sartorius a discharge similar to that produced by an L6 volley. The discharge to an L6 volley was facilitated by an L7 volley preceding at intervals up to *ca.* 15 msec. At longer intervals the discharge to the L6 volley was inhibited.

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ACTION POTENTIAL AND ENZYME ACTIVITY IN THE ELECTRIC ORGAN OF *ELECTROPHORUS ELECTRICUS* (LINNAEUS)

I. CHOLINE ESTERASE AND RESPIRATION

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INTRODUCTION

IT IS GENERALLY accepted that the discharge of electric organs is fundamentally identical in its nature with the action potential of ordinary nerves, the high voltage being caused by the arrangement of the electric plates in series. The close relationship between the E. M. F. of the discharge and the activity of choline esterase in electric organs suggests that acetylcholine metabolism is intrinsically connected with the action potential. This new concept of the role of acetylcholine is strongly supported by several other facts and has recently been described (4, 5, 6).

The parallelism between E. M. F. and enzyme activity first became obvious when, in different species of electric fishes, V per cm. and number of plates per cm. were compared with the concentration of choline esterase (4). It was next shown that the concentration of choline esterase (Ch.E.) in the electric organ of *Electrophorus electricus* decreases from the head to the caudal end in an S-shaped curve and that this curve appears to be similar to that in this organ for the V per cm. and number of plates per cm. (5). The electrical measurements were, however, made on other specimens than the chemical determinations. The connection of the action potential with a chemical reaction is obviously of interest and it appears therefore desirable to establish the relationship in quantitative terms, to compare it with other electrical quantities (resistance, current, power) and to determine what other chemical reactions may be connected with the action potential. In ordinary nerves the electrical changes occurring during activity are small and rapid, the E. M. F. of the action potential being only of the order of millivolts and its duration of the order of milliseconds. These changes and their rate are well within the range of electrical methods, but chemical methods available make it difficult to correlate chemical reactions with electrical phenomena. In the electric organ of *Electrophorus electricus* the voltage of the discharge is high and the differences between the different sections are great. It offers particularly favorable material for studies on the mechanism of nerve activity, especially of chemical reactions involved in electrical changes occurring during nerve activity.

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Such investigations have now been initiated. This paper presents observations on voltage, amperage, resistance and Ch.E. activity on single specimens. Histological findings are presented and respiratory rates are compared with Ch.E. concentration.

METHODS

a. Electrical measurements. The fish was removed from the water and the excess moisture allowed to drain from the skin, which remained, however, wet enough to insure good electrical contact. Voltages were measured with a cathode-ray oscillograph drawing no appreciable current. The terminals of the oscillograph were joined to electrodes which were strips of aluminum 1 cm. wide set transversely in a wooden trough in which the fish was laid. These electrodes made contact at desired positions along the electric organs, through several square centimeters of wet skin next to the organs. A calibrated variable resistance could be connected between the electrodes to draw current from the organs during the discharge.

A set of measurements with one position of the electrodes was made in about three minutes, during which the fish was gently prodded to make it discharge at a frequency high enough for convenient measurement but not high enough to produce rapid fatigue. The discharges of the large organs and the bundles of Sachs could be differentiated by their characteristics described elsewhere (1). The discharge of the bundles of Sachs was generally obtained with a slighter stimulus than that of the large organ; often no stimulus was required. After each set of measuremts the fish was returned to the water for 15 minutes or longer before another set was made with different positions of the electrodes.

The recorded voltage was always the peak voltage of the discharge. It was noted visually on the oscillograph calibrated with a measured voltage. When precautions against fatigue were taken as described, it was found that for any given position of the electrodes and any given value of the variable resistance there was a recurrent peak voltage. Voltages higher and lower than this value were also observed. The lower values occurred more often than the higher; consequently the recorded recurrent voltage was generally somewhat above the average voltage. Estimates of the recurrent voltage by different observers would agree within 5 or 10 per cent. In a set of readings with one position of the electrodes, peak voltages were recorded with different values of the variable resistance. The first reading of the set, and also the last, for a check, were made with the circuit open, so that no current was drawn outside the body of the fish. The readings between were made with decreasing values of the resistance so chosen that with the lowest resistance the peak voltage was about one-fourth of that on open circuit.

b. Determination of choline esterase. The activity of choline esterase was determined with the Barcroft-Warburg manometric method (5). The following solution was used as medium: to 100 cc. of 0.04 molar $MgCl_2$ 20 cc. of 0.15 molar bicarbonate were added. Since the rate of hydrolysis at this Mg concentration[1] is as high as with Ringer concentration this medium appeared preferable. It not only can be more quickly prepared, but can be kept for a long time since no precipitation occurs.

The importance of thorough homogeneous grinding of the highly active tissue has been emphasized (5). Since a large number of determinations had to be made in the shortest possible time, mechanical grinding with a homogenizer (7) has been used except in the experiments with specimens No. 1 and 5, where the tissue was ground by hand. The mechanical grinding has to be done with great care. Silicate has to be added. The experiments and controls made on specimen No. 2 indicate that the pieces should not be too great (see below p. 506). After grinding the suspension was diluted, and the amounts of tissue used per vessel generally did not exceed 0.5–1.0 mg. according to the enzyme activity for reasons previously discussed. Only in the experiments with the bundle of Sachs larger amounts were taken. Weighing and manometric readings were carried out in the same way as before (5).

c. Respiration. Oxygen uptake was measured by the usual Barcroft-Warburg manometric method. 0.1 molar phosphate buffer of pH 7.4 was taken as a medium. If the substrate was glucose or pyruvate, slices of finely minced tissue were used in conical vessels,

[1] Nachmansohn. Unpublished experiments

and 0.2 cc. of 8 per cent KOH was put in the center well. It is difficult to cut the gelatinous tissue in thin slices, but since respiration is extremely small, diffusion of O_2 through the finely minced tissue most probably is not the limiting factor.

The oxidation of succinate was measured with homogenized tissue. Square vessels with a side bulb were used. The substrate concentration was 0.15 molar in 0.1 molar phosphate buffer of pH 7.4. In the side bulb were put 0.1 cc. of cytochrome c solution, containing about 1.5 mg. of cytochrome c in phosphate buffer. The cytochrome was prepared according to the method of Keilin and Hartree. It was added after thermoequilibrium was reached. Controls were run without succinate, and only the difference between the oxygen uptake of control and experiment recorded.

Succinic dehydrogenase was determined with the manometric method according to the principle used by Quastel and Wheatley (8). Ferricyanide is reduced by the hydrogen of succinic acid. For one reduced molecule of ferricyanide one molecule of acid is formed and in bicarbonate solution this gives rise to one molecule of CO_2. The details of the method as used in these experiments were similar to those used by Nachmansohn and Steinbach (6). The concentration of sodium succinate was 0.15 molar.

d. Histological preparations. All fishes were killed by freezing in dry ice. Immediately after death, blocks were cut from the frozen organ. The length of the blocks in the direction head to caudal end was usually exactly one cm. From specimen No. 2 blocks 2 cm. long were cut. The blocks were cut out in most cases close to the center of the segment of which the voltage had been determined. Serial sections were made using Zenker fixation and Mallory aniline blue stain.

RESULTS

The experiments were carried out on 4 specimens, two small and two medium sized. On a fifth specimen, of medium size, only Ch.E. determinations were made. But since the total maximal discharge was known and differs from that of the two other medium sized specimens, the description appears useful and has been added.

The lengths of the specimens and their organs were as follows:

Specimen No.	Total length	Length of the electric organ
	cm.	cm.
1	51	41
2	57	47
3	112	92
4	114	94
5	105	87

A. *Electrical data*

A complete series of measurements on the discharge of the large organ made on specimen No. 4 is recorded in Table 1. In the first set of the series with the electrodes placed at 0 and 10 cm. distance from the head end of the electric organ there was reason to think that the fish had become fatigued during its exertions, muscular and electrical, while it was being removed from the tank and placed in the trough. This set was repeated therefore after the fish had rested half an hour in the water, as well as at the end of the series.

The recorded values of the resistance were known from the calibration. Those of the peak voltage were measured as already described. The current

Table 1. Measurements of the discharge of the large organ. Specimen No. 4. Ω = ohms, V = volts, A = amperes. ∞ = open circuit.

Position of electrodes	0–10 cm.		0–10 cm.		20–30 cm.		40–50 cm.		50–60 cm.		0–10 cm.	
Ω	V	A	V	A	V	A	V	A	V	A	V	A
∞	64	0	65	0	38.6	0	11.5	0	8.3	0	59	0
800	60	0.075	59	0.074	34.5	0.043	10.0	0.0125	7.1	0.0089	52	0.065
400	47	0.118	48	0.120	31.2	0.078	7.9	0.0198	6.4	0.0160	46	0.115
200	27	0.135	42	0.210	26.0	0.130	6.0	0.030	5.1	0.0255	37	0.185
100	18	0.180	31	0.310	18.1	0.181	3.9	0.039	3.7	0.037	25	0.250
50	7	0.140	17	0.340	10.1	0.202	2.4	0.048	2.4	0.048	16	0.320
∞	56	0	59	0	38.6	0	12.0	0	8.6	0	57	0

produced in the resistance at the peak of the discharge is the quotient of the voltage by the resistance.

The data are shown graphically in Fig. 1, in which the voltage is plotted as ordinate against the current as abscissa and a straight line is drawn through the plotted points, as was done by Coates and Cox (2) on *Torpedo occidentalis*. The intercept of this line on the axis of voltage is taken as the maximum voltage for each segment of the organ and the intercept on the

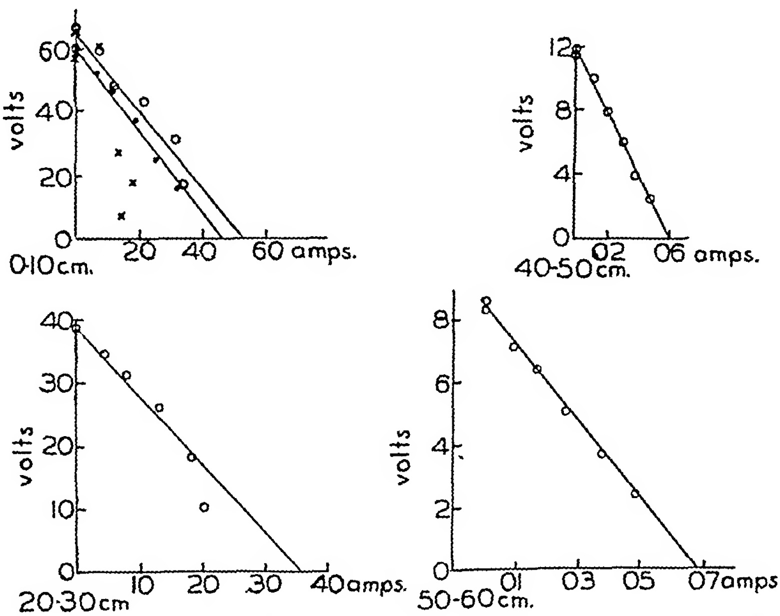

FIG. 1. Voltage of the discharge of the electric organ recorded with four different positions of electrodes. Abscissae: amperes. Ordinates: volts.

axis of current as the maximum current. All the values of maximum voltage and current recorded in Table 2 were similarly obtained. The values of resistance recorded in this table are the quotients of the maximum voltage by the maximum current.

The higher the resistance, the steeper is the line through the points.

Table 2. Electrical data recorded on specimens No. 1–4.

Specimen No.	1. Position of electrodes (cm)	2. Maximum voltage (V)	3. Maximum current (A)	4. Resistance (Ω)	5. Voltage per cm.	6. No. of electroplaxes per cm	7. mV per electroplax	8. QCh.E. average	
1.	1–6	95	0.148	640	19.0	133	145	492	
	11–16	79	0.104	760	15.8	119	133	415	
	16–21	44	0.059	750	8.8	72	119	280	
	21–26	20.4	0.019	1060	4.1	—	—	150	
2.	1–6	126	0.307	410	23.9	146	164	455	
		113	0.257	440					
	11–16	92	0.278	330	18.4	131	140	407	
	21–26	45	0.128	350	9.0	—	—	320	
	31–36	12	0.021	570	2.4	—	—	225	
3.	0–10	60	0.374	160	6.7	—	—	145	
	20–30	34	0.243	140	3.8	48	79	81	
	40–50	9 2	0.077	120	1.0	—	—	60	
	50–60	7.0	0.041	170	0.8	—	—	—	
4.	0–10	65	0.52	125	6.9	65	106	110	
		59	0.46	128					
	20–30	39	0.36	108	4.3	40	107	81	
	40–50	11.9	0.060	198	1.3	—	—	51.5	
	50–60	8.5	0.068	125	0.9	—	—	—	
	30–40	7.3	0.028	260	0.81	—	—	—	b.o.S.
	40–50	8.6	0.043	200	0.91	—	—	21	b.o.S.
		7.8	0.035	220					
	60–70	8.1	0.051	160	0.90	8	112	23	b.o.S.

With fatigue the points lie on a steeper line, so that at least one indication of fatigue is an increased resistance. With marked fatigue the points may fall so irregularly that no line can be drawn. In this case also the readings are not so well reproduced, so that the points themselves are uncertain. Such a set of points is shown by the crosses of Fig. 1 (a), plotted from the first set of readings of Table 1. The second set is shown by the open circles and the third set by the closed circles. The points of these two sets fall not far from a common line, but it seemed better to draw one line for each set. Two values

were recorded therefore for the maximum voltage, maximum current, and resistance (see Table 3, specimen No. 4). The mean of the two values of maximum voltage was used to obtain the voltage per cm. In some of the observations on other fish, when two sets of measurements were made on the same segment of the organs, and the values of voltage and current were plotted as in Fig. 1, a single straight line served equally well to describe

Table 3. Concentration of choline esterase at different sections of the electric organ.

Specimen No. 2

Distance from ant. end (cm.)	Fresh weight (mg.)	QCh.E. single	QCh.E. average
3	311.0	435 470 450 465	*455*
11	238.0	414 435	*425*
18	158.5	351 343 391	*362*
26.5	128.5	320 300	*310*
31	17.8	250 239	*245*

Specimen No. 4

	Distance from ant. end (cm.)	Fresh weight (mg.)	QCh.E. single	QCh.E. average	
Large organ	5	59.0 42.0 34.0	100, 88, 85 100, 98 139	91.0 99.0 139.0	*110.0*
	25	99.8 36.0 51.5	59, 72, 76 121.5, 110 62, 55	69.0 116.0 58.5	*81.0*
	35	66.4 63.7 40.8	54.5 65.0 85.0	— — —	*68.0*
	45	65.0 43.3 71.7	50.4, 51 33.5, 37.5 82, 53	50.7 35.5 68.0	*51.5*
Bundle of Sachs	45	74.3 53.5	21.6 20.0	— —	*20.8*
	60	108.0	18.7, 27.8	23.2	*23.2*
	82	87.4 77.0	21.5 27.3	— —	*24.4*

either set. In these cases only one value was recorded for the maximum voltage, maximum current, and resistance.

The current obtained as the quotient of the voltage by the external resistance is, of course, only that flowing in the resistance and does not include any current flowing through the tissue which surrounds the organ. If there were no such leakage of current, then the computed current actually would be equal to the current flowing through the electric organ, the maximum voltage would be the electromotive force of the segment of the organ included between the electrodes, and the value of the resistance found as the quotient of the maximum voltage by the maximum current would be the actual resistance of this segment of the organ at the peak of the discharge.

It was found by Cox and Coates that a number of measurements under varied conditions could be fairly well represented by treating the current flowing through the tissue around the organ as if it flowed in a single resistance joined in parallel with the external resistance. They also found evidence that the resistance of the organ itself drops sharply during the discharge, a conclusion which conforms well to Bernstein's hypothesis that the discharge occurs when one boundary of each electroplax becomes permeable to an electric current in the direction in which the discharge passes through the organ.

To estimate the resistance of the tissue around the organ and thus to reckon its effect in reducing the external maximum voltage below the electromotive force, the resistance of the part of the fish between the electrodes was measured with an ohmmeter while the fish was resting in the trough without discharging. Resistances were found as follows for the several segments; 0–10 cm., 2000 ohms; 20–30 cm., 2200 ohms; 40–50 cm., 2400 ohms; 50–60 cm., 2200 ohms. These values might be taken as those of the resistance of the tissue around the organ on the assumption that the organ itself is practically non-conducting during the discharge. With these values the scheme of Cox and Coates could be used to compute the electromotive force and corrected values of the maximum current flowing in the segment of the organ and its resistance at the peak of the discharge. The method of the correction, however, is admittedly crude and its amount in any case did not seem important. Consequently the correction was not made, and the values of the voltage per cm. correlated with the QCh.E. have been taken from the measured maximum voltage rather than from the estimated electromotive force, which would have been always somewhat higher.

B. *Concentration of choline esterase and E. M. F.*

The tissue taken for the determinations of Ch.E. concentration was cut out as close as possible to the center of each segment, for which the electrical data were recorded. Since all the fish were prepared by freezing in dry ice and kept in it until the end of the experiments there was no danger of loss of water which may increase the QCh.E. values (5).

Figure 2 shows the data obtained with specimen No. 1. It demonstrates the close parallelism between V per cm. and QCh.E. Specimen No. 2 was about the same size as the first. Both Ch.E. concentration and E. M. F. are of the same order of magnitude as in the first fish (see Tables 2 and 3). The decrease of voltage is however steeper than in the first fish and greater than would be expected from the fall of Ch.E. concentration which is slightly smaller than in the first specimen. Two remarks, however, have to be made: This was the first specimen where the mechanical grinding was used for the determinations of Ch.E. No silicate was added and pieces of tissue of 150–300 mg. fresh weight and handgrinding were about 10–15 per cent higher than the average values obtained from the large pieces ground with the homogenizer. The average QCh.E. values from three small pieces at 11 cm. distance from the

anterior end of the organ was 459 instead of 425 obtained with the large piece ground mechanically. From three pieces at 18 cm. distance the QCh.E. was 407 instead of 362. It appears probable that the value at 3 cm. distance was actually around 500. The pieces of the caudal end were smaller and the

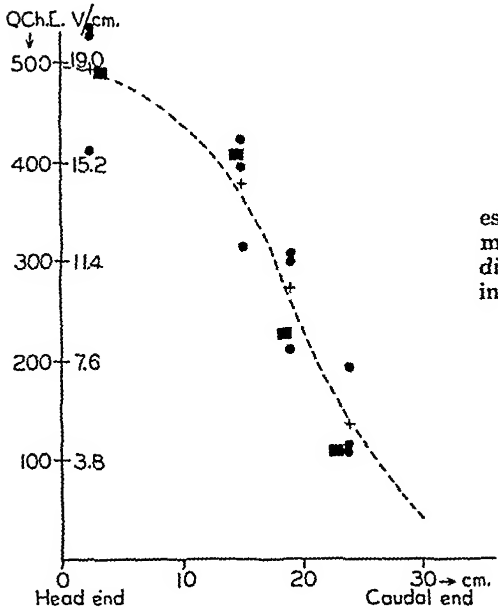

FIG. 2. Action potential and choline esterase activity in the electric organ. Specimen No. 1. Length of fish 51 cm. Abscissae: distance from the anterior end of the organ in cm. Ordinates: QCh.E. and V/cm.
● average QCh.E. from a single piece of tissue
+ average QCh.E. values from pieces of the same section
■ V/cm.

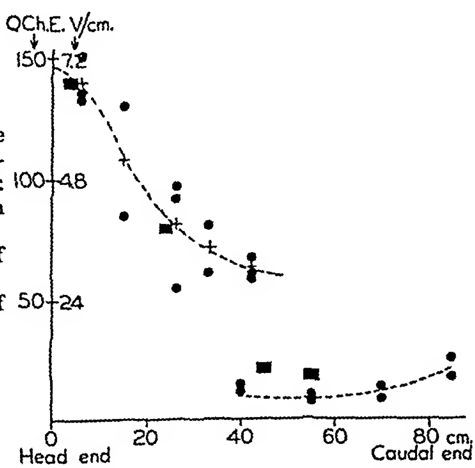

FIG. 3. Action potential and choline esterase activity in the electric organ. Specimen No. 3. Length of fish 112 cm. Abscissae: distance from the anterior end of the organ in cm. Ordinates: QCh.E. and V/cm.
● average QCh.E. from a single piece of tissue
+ average QCh.E. values from pieces of the same section
■ V/cm.

determinations therefore probably more correct. In the experiments with specimens 3 and 4 the pieces were small (see tissue weights of specimen No. 4, Table 3). The grinding was carried out more thoroughly and silicate was added.

As far as the electrical data are concerned it must be noted that this

specimen was not in excellent condition when taken for the experiments. It had vomited repeatedly, refused food for several days, was sluggish and inactive. It seems possible that the correlation was impaired by the poor physical condition of the fish. Since Ch.E. is a stable enzyme the curve of voltage can drop below that of the enzyme concentration in case the discharge is not optimal.

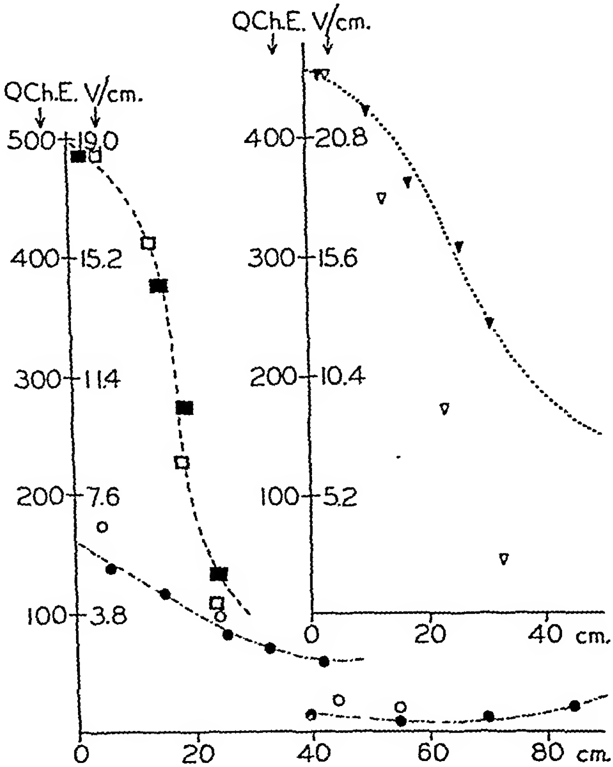

FIG. 4. Action potential and choline esterase activity in the electric organs of three specimens. Abscissae: distance from the anterior end of the organ in cm. Ordinates: QCh.E. and V/cm.
■ QCh.E., □ V/cm. of specimen No. 1
▼ " ▽ " " No. 2
● " ○ " " No. 3

The voltage per cm. and the concentration of Ch.E. are lower in the large than in the small specimens (5). It was however not certain in these experiments whether in absolute amounts there is a correlation between voltage and enzyme concentration if different sizes are compared. The following experiments seem to indicate that there does exist a parallelism between voltage and enzyme concentration even in absolute amounts and independent of the size of the specimen.

Specimen No. 3 (Fig. 6) was a good medium sized fish. Fig. 3 shows the

data obtained in the same way as those of the first specimen in Fig. 2. In the bundle of Sachs the QCh.E. values drawn in a separate curve are much lower than in the main organ. But the concentration seems to be rather equal in its different sections. The values obtained with specimen No. 4 which had about the same size as No. 3 are practically identical with those of the third specimen (see Tables 2 and 3). On this specimen measurements were carried out on three different segments, again in agreement with the even distribution of Ch.E. in this organ.

Figure 4 demonstrates how closely, even in absolute amounts, the voltage parallels the Ch.E. activity. The data obtained on all specimens are summarized. Only No. 4 has been omitted since the figures are so close to those

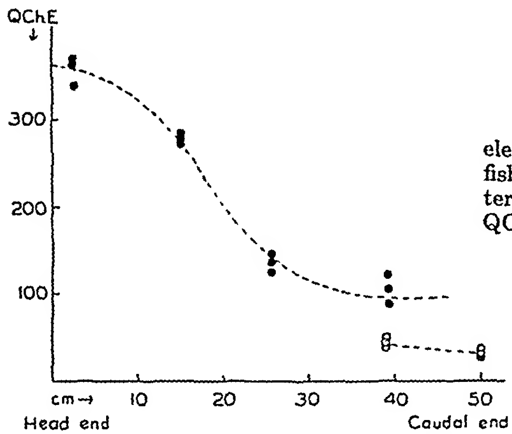

FIG. 5. Choline esterase activity in the electric organ. Specimen No. 5. Length of fish 105 cm. Abscissae: distance from the anterior end of the organ in cm. Ordinates: QCh.E.
● average QCh.E. from a single piece of tissue

of No. 3 that they would interfere with each other. No. 2 has been drawn separately in order to make clearer the difference from No. 1, since otherwise again the two curves would interfere.

Specimen No. 5 (Fig. 7) was found dead in the Aquarium one morning. It was put immediately on ice and all Ch.E. determinations were made on the following day. It is not probable that under these conditions there was any measurable loss of water in such a short time. Figure 5 gives the values obtained. They are considerably higher than in the two other medium sized specimens, ranging from 356 (mean value of the first section, 2.5 cm. from the anterior end of the electric organ) to 106 (mean value 38 cm. from the anterior end). In the bundle of Sachs the values are 44 and 31. Even if the values are a few per cent too high, due to loss of water, it is interesting to note that they are more than twice as high as in the two other medium sized specimens. The total maximum voltage of this fish was 420 volts, as compared with less than 200 volts in specimens No. 3 and 4 (Fig. 6). This again is in good agreement with the assumption that even in absolute amounts voltage and Ch.E. activity run parallel.

The number of electroplaxes per cm. is known for many segments from the histological preparations (see paragraph D). The voltage per electroplax

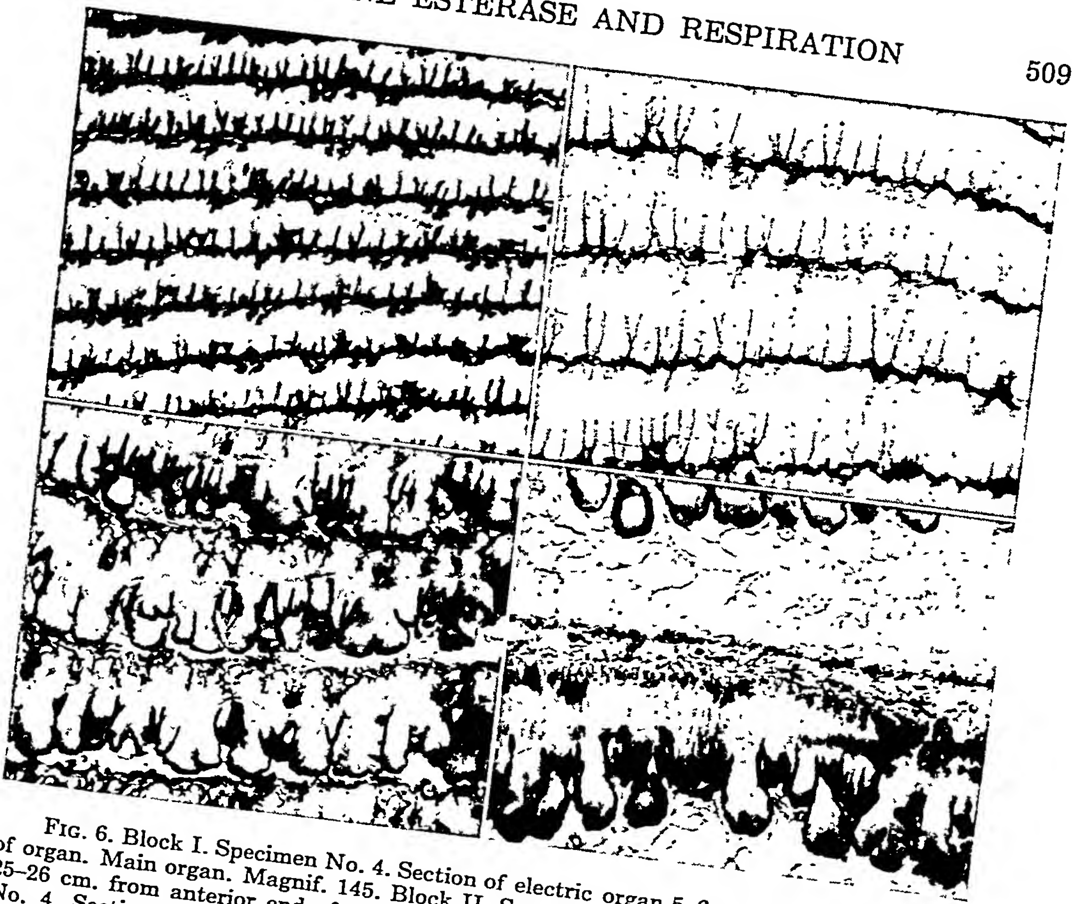

FIG. 6. Block I. Specimen No. 4. Section of electric organ 5–6 cm. from anterior end of organ. Main organ. Magnif. 145. Block II. Specimen No. 4. Section of electric organ 25–26 cm. from anterior end of organ. Main organ. Magnif. 145. Block III. Specimen No. 4. Section of electric organ 40–41 cm. from anterior end of organ. Main organ. Magnif. 145. Block IV. Specimen No. 4. Section of electric organ 55–56 cm. from anterior end of organ. Bundle of Sachs. Magnif. 145.

has been calculated in those cases where the blocks for histological preparations were taken from the center or close to the center of the segment of which the electrical data were recorded. Not enough data were available to permit an interpolation. As far as can be judged from the few data obtained on the two small specimens (Table 2, column 7) the voltage per electroplax seems to be not equal in all segments but to decrease from the head to the caudal end of the organ. The decrease of the E.M.F. may therefore depend not only on the number of plates per cm. but also on the voltage per electroplax.

In column 8 of the same table the QCh.E. values are indicated. In this case if the tissue was not cut out from the center of the segment, the values were interpolated from the curve since the data appear sufficient for such interpolation.

The voltage per electroplax in the larger specimen could be calculated only for a few cases. Both voltage and Ch.E. concentration are lower than

in the small specimens (see Table 2, column 7 and 8). Not enough data are available to permit any conclusion.

C. *Respiration*

Oxidation is the ultimate source of all energy used in living cells. The discharge of the electric organ yields a measurable amount of electric energy and, for nerve, it has been shown that activity is connected with heat

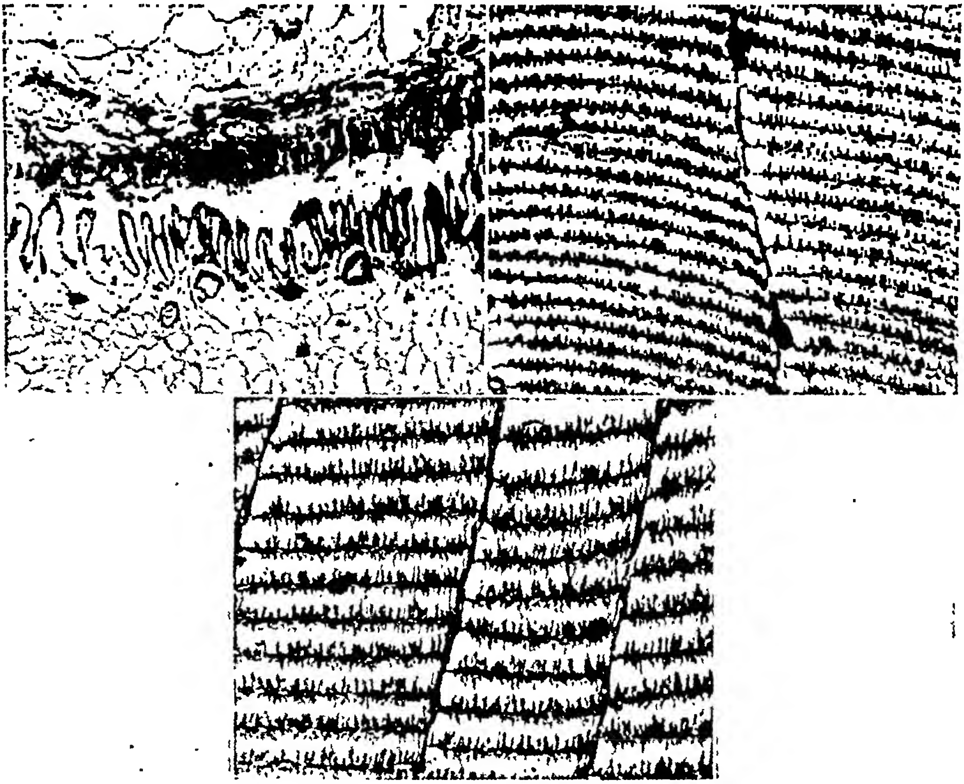

FIG. 7. Block V. Specimen No. 4. Section of electric organ 70–71 cm. from anterior end of organ. Bundle of Sachs. Magnif. 145. Block VI. Specimen No. 2. Section of electric organ 3.5–5.5 cm. from anterior end of the organ. Magnif. 145. Block VII. Specimen No. 2. Section of electric organ 18.5–20.5 cm. from anterior end of organ. Magnif. 145.

production and extra oxygen uptake. But, as recently discussed by Nachmansohn and Steinbach (6), it is improbable that oxidation is directly connected with the action potential, its energy being used only in the recovery period. Experiments by these investigators on the giant axon of squids suggest that the respiratory enzymes are chiefly concentrated in the axoplasm in contrast to Ch.E. which is localized at or near the surface of the axon.

The respiration of the electric tissue has been studied at different sec-

tions and with different substrates (pyruvate, glucose and succinate). With succinate both the dehydrogenase and the oxidizing system were examined. The tissue was taken by biopsy, since respiration is greatly impaired in fishes killed by freezing in dry ice. The fish withstands the operation well. From one fish 19 biopsies were made and it was still in rather good condition. Succinic dehydrogenase is not damaged by the freezing.

The respiration of the electric tissue is low. Table 4 shows the results

Table 4. Succinic oxidizing system and succinic dehydrogenase at different sections of the electric organ.

Succinic oxidizing system

Specimen No	Distance from ant end (cm)	Fresh weight (mg)	min obsery	cmm O_2 absorb	cmm O_2 per 100 mg in 60 min corr	Q_{O_2} s	Q_{O_2} a
1	2.5	74.0	90	7.6	6.1		*−0.51*
	15.0	73.0	90	5.8	5.6		*−0.47*
	20.0	86.0	90	7.0	5.1		*−0.43*
3	10	219	60	8.8	3.8	−0.48	*−0.37*
		143	85	8.1	2.0	−0.25	
	30	190	85	17.6	3.1	−0.39	*−0.37*
		160	85	16.2	3.2	−0.40	
		202	60	4.5	2.3	−0.33	
	50	194	90	8.1	0.9	−0.11	*−0.30*
		226	90	17.7	4.3	−0.41	
		176	90	16.3	3.3	−0.54	
		216	90	3.1	1.0	−0.13	

Succinic dehydrogenase

Specimen No	Distance from ant end (cm)	Fresh weight (mg)	min obs	cmm CO_2 per 100 mg corr	μg succ ac per 100 mg in 60 min
1	2.5	62.4	75	44.2	*93.5*
	15.0	65.0	75	43.5	*92.0*
	23.0	59.7	90	60.6	*106.0*
3	10	111.0	110	25.8	*42*
				25.7	
				27.9	
	30	110.0	120	28.2	*36*
				25.0	
				28.2	

obtained with succinate, table 5 with pyruvate and glucose. The distance from the anterior end of the organ from which the tissue was cut did not measurably alter values. Even in the bundle of Sachs the Q_{O_2} values are the same as in the main organ.

D. *Histological preparations*

Not only the number of electroplaxes per cm. varied in the different sections but their aspect too changed. A number of these preparations were photographed to show the changes of the voltaic pile and some of them are reproduced (see photos 1–7). Since the voltage per electroplax in the small specimen is slightly higher than in the larger fishes, the pictures illustrate why the voltaic pile is so much stronger there. They also show how well the anatomical structure corresponds to the chemical and electrical data.

The number of plates was calculated as the mean value of several serial sections of the same block. The results are summarized in Table 7. Each

figure of the third column is calculated from one serial section. In the first two blocks of specimen No. 2, where two rows are given, the result of a second calculation of the same section is given in the second row. The figures show that two calculations of the same serial section agree satisfactorily, whereas the variations, obtained with different sections of the

Table 5. Respiration of the electric organ at different sections between head and caudal end, with glucose and pyruvate as substrate. The O_2 uptake was observed in the experiments with glucose during 90 min. on specimen No. 3, during 60–80 min. on specimen No. 4, and those with pyruvate during 70 min. At 50(58) cm. bundle of Sachs.

Specimen No.	Distance from ant. end (cm.)	Tissue fresh weight (mg.)	Q_{O_2} single	Q_{O_2} average
		Glucose		
3	10	198 186	−0.36 −0.22	*−0.29*
	30	200 146	−0.27 −0.21	*−0.24*
	50	223 226 196 213	−0.54 −0.26 −0.41 −0.15	*−0.34*
4	10	278 240 297 217	−0.58 −0.31 −0.39 −0.41	*−0.42*
	30	223 284 236	−0.27 −0.30 −0.43	*−0.33*
	58	255 252 265	−0.39 −0.51 −0.48	*−0.46*
		Pyruvate		
3	10 30 50	217 195 202		*−0.32* *−0.28* *−0.33*

same block, were more marked. In one case only, in the third block of specimen No. 2, there are two figures, 245 and 246, which are considerably higher than all the others. It may be that these two sections belong to the main organ whereas the other sections belong to the bundle of Sachs.

Discussion

The experiments offer new evidence for the close correlation between

Ch.E. activity and the action potential. V per cm. and QCh.E. run parallel, not only if measured on the same specimen, but even if compared in fishes of different size and with considerable differences of the E. M. F. of the discharge. The parallelism appears therefore to be so close, even in absolute amounts, that the enzyme concentration may be predicted from the voltage and *vice versa* with good approximation.

The voltage per electroplax has been measured only in a few cases. No

Table 6. Number of electroplaxes in the different sections of the electric organs of the four specimens examined.

Specimen No.	D	Number of electroplaxes counted	Average per cm.	
1	3–4	128, 130, 142	133	
	12–13	111, 117, 114, 126, 125	119	
	16.5–17.5	63, 75, 78, 71	72	
2	3.5–5.5	281, 284, 280, 297, 306 286, 296, 289, 299, 302	146	
	11.5–13.5	260, 256, 251, 264, 279 251, 260, 253, 268, 282	131	
	18.5–20.5	182, 181, 185, 216, 217, 245, 246	105	
	25.5–27.5	87, 120, 104 90, 115	48	
3	25.5–26.5	53, 45, 49, 52, 51, 48, 39	48	
	45.5–46.5	5, 5, 5, 7, 8, 10, 9, 10	7	b.o.S.
	55.5–56.5	6, 5, 8, 8, 8, 9, 8	7	
4	5–6	76, 71, 66, 65, 58, 54	65	
	25–26	40, 41, 40, 40	40	
	40–41	26, 24, 18, 16	21	
	55–56	12, 10, 8, 7	9	b.o.S.
	70–71	9, 8, 7, 8, 7	8	

final conclusions are therefore possible. It seems that the voltage per plate is not uniform. It would be desirable to know whether the concentration of enzyme per electroplax changes correspondingly. From the few data available it appears as if there may be some relationship between the concentration of enzyme and voltage per electroplax. However, the parallelism is not as well established as between V per cm. and QCh.E. More data are necessary to elucidate this question.

The concept that the activity of Ch.E. is intrinsically connected with the discharge is emphasized by the fact that this correlation appears to be specific. Respiration which is also necessary for nerve activity, does not vary from the head to the caudal end in sharp contrast to the variations of the QCh.E. values. Other enzymes and substances, examined so far, which will be described in following papers, do not show any parallelism with voltage.

It would be interesting to measure the resting respiration of the intact organ and to compare it with the respiration after activity. It is probable that after activity the oxygen uptake rises and it is possible that it rises somewhat more near the head than at the caudal end. In stimulated nerves the oxygen uptake is about twice as high as in the resting nerve. But the following consideration can be applied. Oxidation is a slow process. In nerve and electric tissue the rate is particularly low. If the QCh.E. is 150–250, 5–10000 times more molecules of ACh can be split per unit of tissue and per unit of time, than molecules of oxygen can be taken up. Even these figures do not indicate the real ratio between the rate of ACh hydrolysis and oxidation. For the QCh.E. values are calculated per unit of weight of tissue whereas it is known that ACh metabolism occurs only at or near the surface of the neurone, that is within a minute fraction of the actual tissue weight. This correction has not to be made for oxidation. Since electrical phenomena occur at a high speed, the high rate of ACh hydrolysis is in itself suggestive, if compared with the low respiration rate and is certainly closer to the discharge than oxidation which may be the last link of a long chain of reactions connected with the action potential.

It will be noticed that it is the voltage per cm. which has been correlated with the QCh.E., whereas the release of ACh may also have to do directly with the alteration of the resistance during the discharge (5). The values of the resistance of the segments of the organ (Table 2) cannot be interpreted without consideration of the cross-sectional area of the segments as well as their lengths. For it is only this specific resistance which might be expected to have some correlation with the chemical reactions of the tissue. The cross sections of the segments have been measured in most of the specimens and the resistance of the organ will be discussed in a following paper. It is at any rate clear that the activity of Ch.E. is closely connected with the electrical activity, both from the results here reported and others to which reference has been made. It is also certain, that a change in the resistance of the tissue is an important feature of the electrical action. Whether the change in resistance is accompanied by a change in E. M. F. and what is the immediate effect of the release of ACh on the electrical quantities are questions on which more experiments are needed.

Summary

1. Voltage, amperage and resistance have been recorded in the electric organ of four specimens of *Electrophorus electricus*, at different sections between head and caudal end. The data were compared with the concentration of Ch.E. at the same segments. V per cm. and QCh.E. run parallel not only in the same fish, but even in fishes of different size in which the E. M. F. of the discharge differs considerably. The experiments offer new evidence for the close relationship between E. M. F. and Ch.E. activity.

2. The respiration has been measured in different sections of the electric organ with different substrates; glucose, pyruvate, and succinate. With

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